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(54) **PLATFORM ANTIBODY COMPOSITIONS**

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(57) **ABSTRACT**

The present invention provides novel compositions of human IgG_2 antibodies comprising a chelating agent. The present invention also provides novel antibody compositions that exhibit improved chemical and/or physical stability. Also provided are methods of treating diseases and conditions with compositions of human IgG_2 antibodies, including inflammatory diseases and neoplasia disorders.

Figure 1

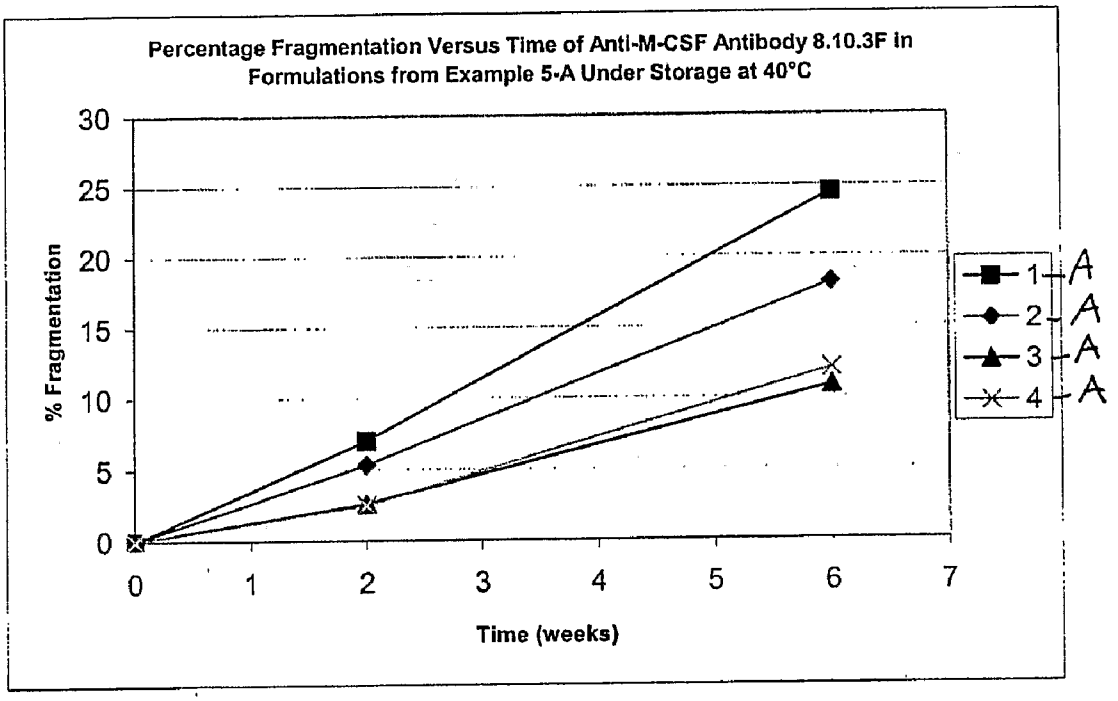

Percentage Fragmentation Versus Time of Anti-M-CSF Antibody 8.10.3F in Formulations from Example 5-A Under Storage at 40°C
% Fragmentation
30
25
20
15
10
5
0
0
1
2
3
4
5
6
7
Time (weeks)
1 A
2 A
3 A
4 A

Figure 2

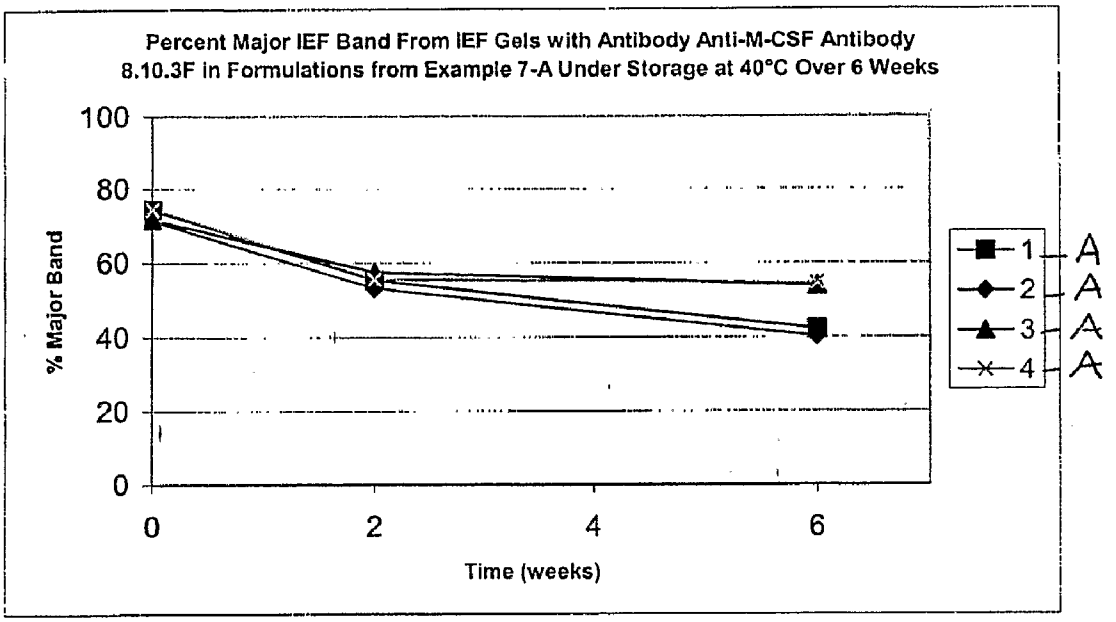

Percent Major IEF Band From IEF Gels with Antibody Anti-M-CSF Antibody 8.10.3F in Formulations from Example 7-A Under Storage at 40°C Over 6 Weeks
% Major Band
100
80
60
40
20
0
0
2
4
6
Time (weeks)
1 A
2 A
3 A
4 A

Figure 3

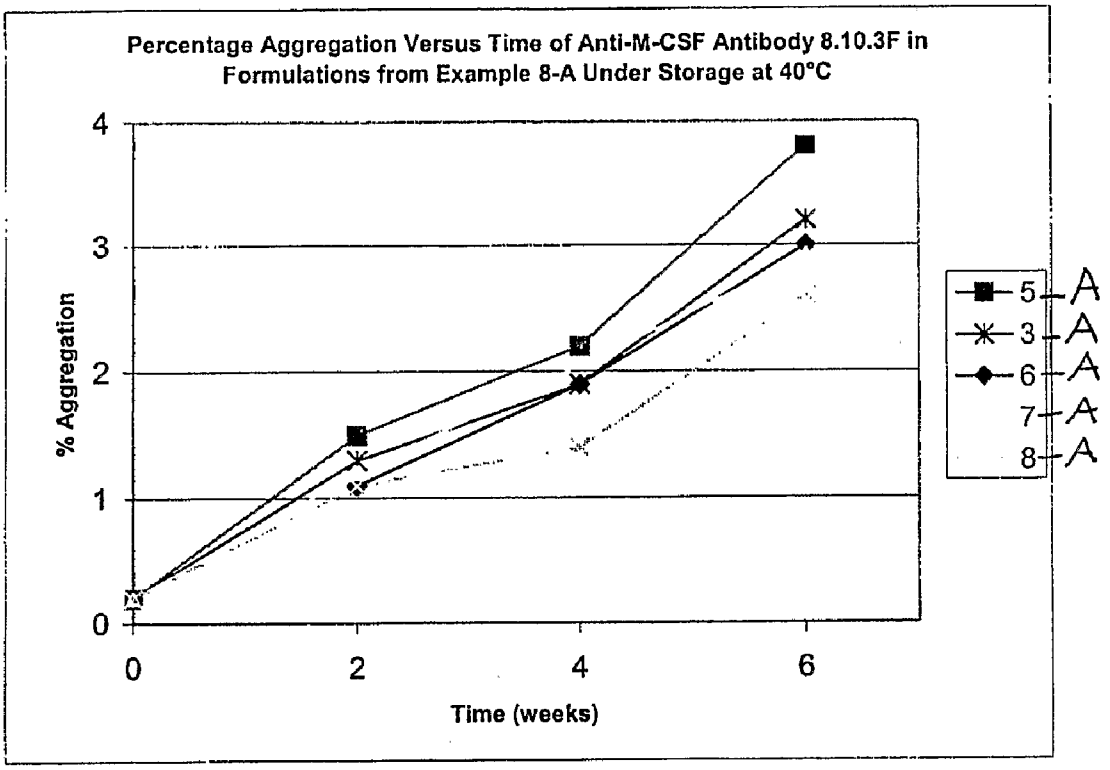

Percentage Aggregation Versus Time of Anti-M-CSF Antibody 8.10.3F in Formulations from Example 8-A Under Storage at 40°C
% Aggregation
0
1
2
3
4
0
2
4
6
Time (weeks)
5 A
3 A
6 A
7 A
8 A

Figure 4

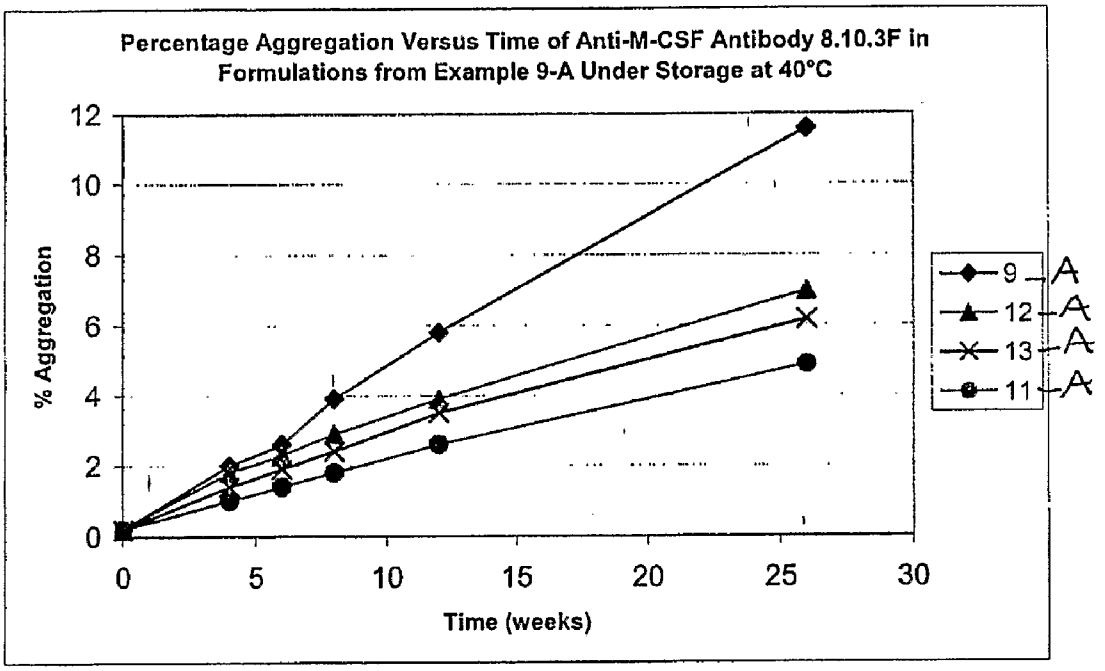

Percentage Aggregation Versus Time of Anti-M-CSF Antibody 8.10.3F in Formulations from Example 9-A Under Storage at 40°C
% Aggregation
0
2
4
6
8
10
12
0
5
10
15
20
25
30
Time (weeks)
9 A
12 A
13 A
11 A

Figure 5

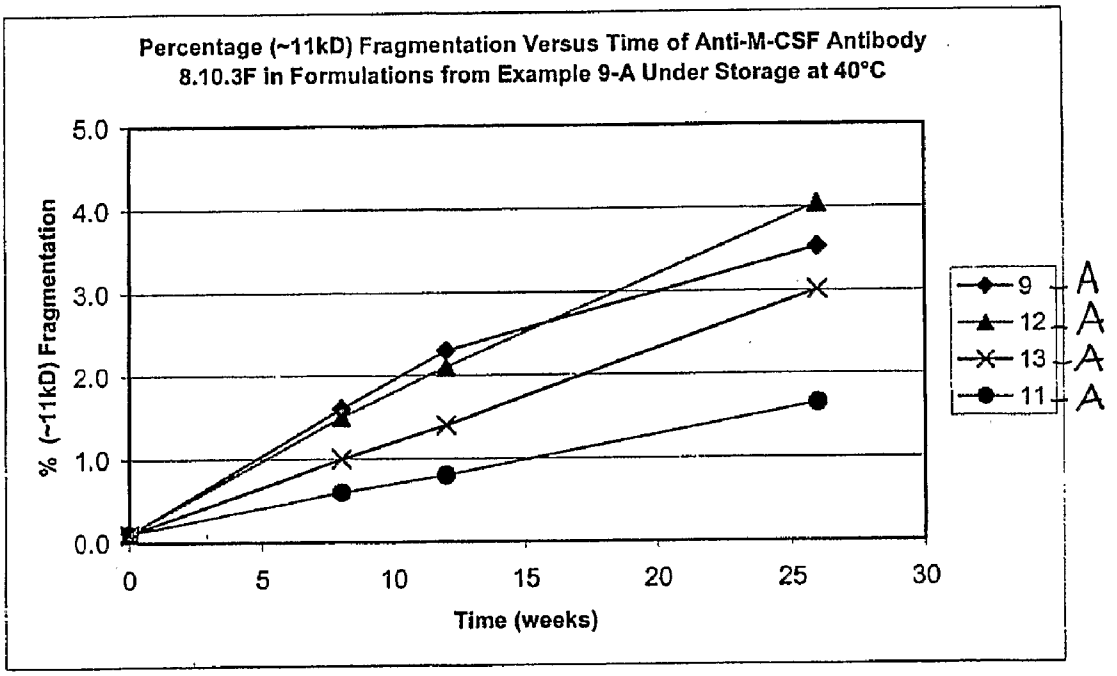

Percentage (~11kD) Fragmentation Versus Time of Anti-M-CSF Antibody 8.10.3F in Formulations from Example 9-A Under Storage at 40°C
% (~11kD) Fragmentation
5.0
4.0
3.0
2.0
1.0
0.0
0
5
10
15
20
25
30
Time (weeks)
9 A
12 A
13 A
11 A

Figure 6

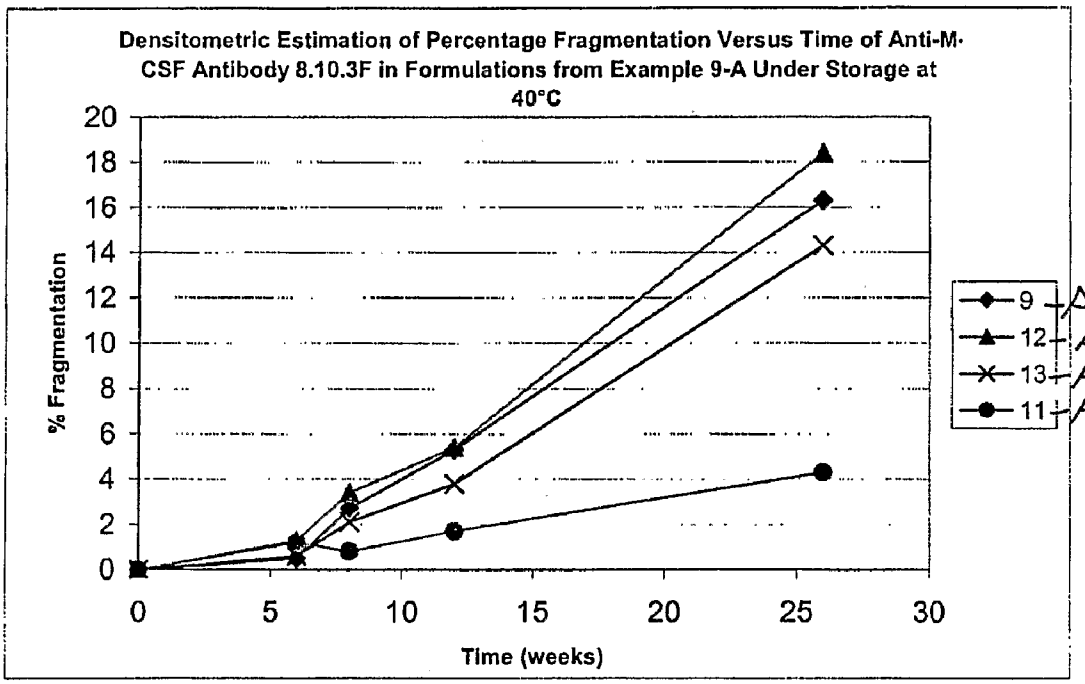

Densitometric Estimation of Percentage Fragmentation Versus Time of Anti-M-CSF Antibody 8.10.3F in Formulations from Example 9-A Under Storage at 40°C
% Fragmentation
Time (weeks)
0
2
4
6
8
10
12
14
16
18
20
0
5
10
15
20
25
30
9-A
12-A
13-A
11-A

Figure 7

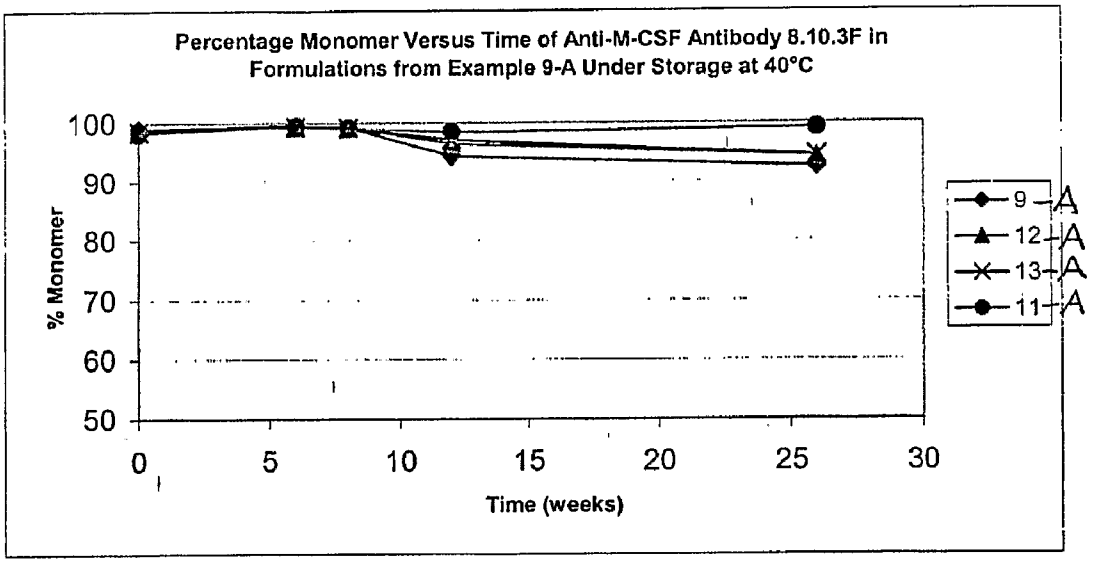

Percentage Monomer Versus Time of Anti-M-CSF Antibody 8.10.3F in Formulations from Example 9-A Under Storage at 40°C
% Monomer
100
90
80
70
60
50
0
5
10
15
20
25
30
Time (weeks)
9-A
12-A
13-A
11-A

Figure 8

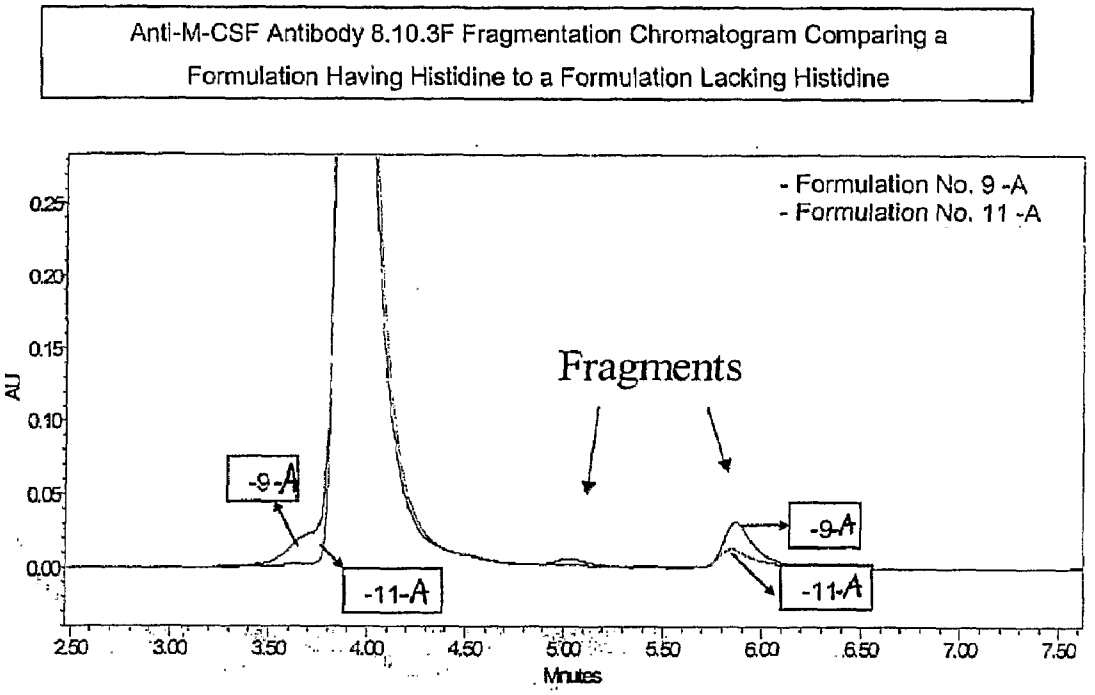

Anti-M-CSF Antibody 8.10.3F Fragmentation Chromatogram Comparing a Formulation Having Histidine to a Formulation Lacking Histidine
- Formulation No. 9 -A
- Formulation No. 11 -A
Fragments
-9-A
-11-A
-9-A
-11-A
AU
0.25
0.20
0.15
0.10
0.05
0.00
2.50
3.00
3.50
4.00
4.50
5.00
5.50
6.00
6.50
7.00
7.50
Minutes

Figure 9

Anti-M-CSF Antibody 8.10.3F
Gel Photograph of Reduced SDS-PAGE Fragmentation Pattern

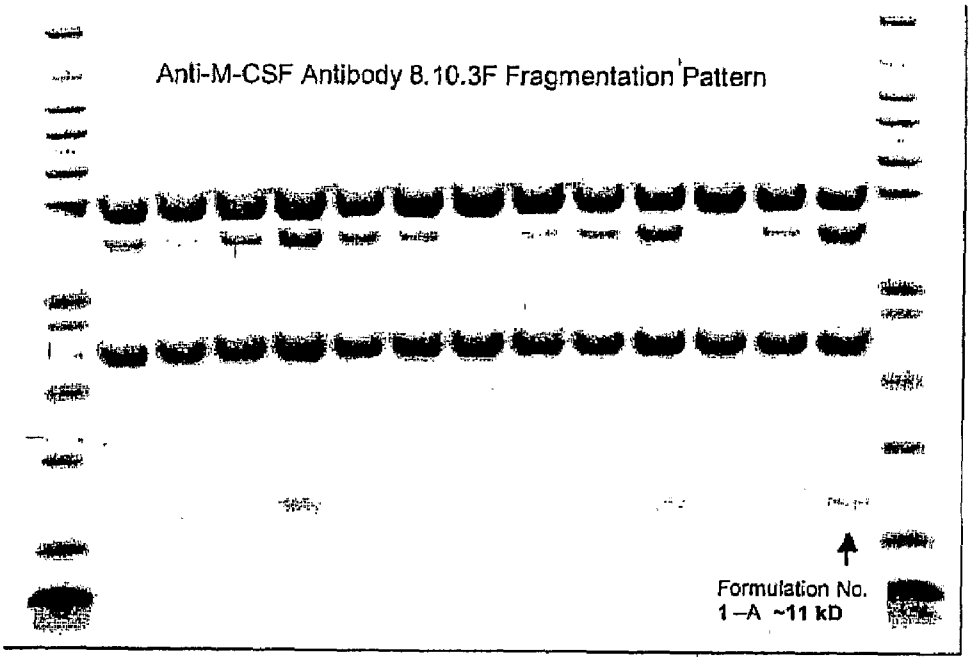


Figure 10

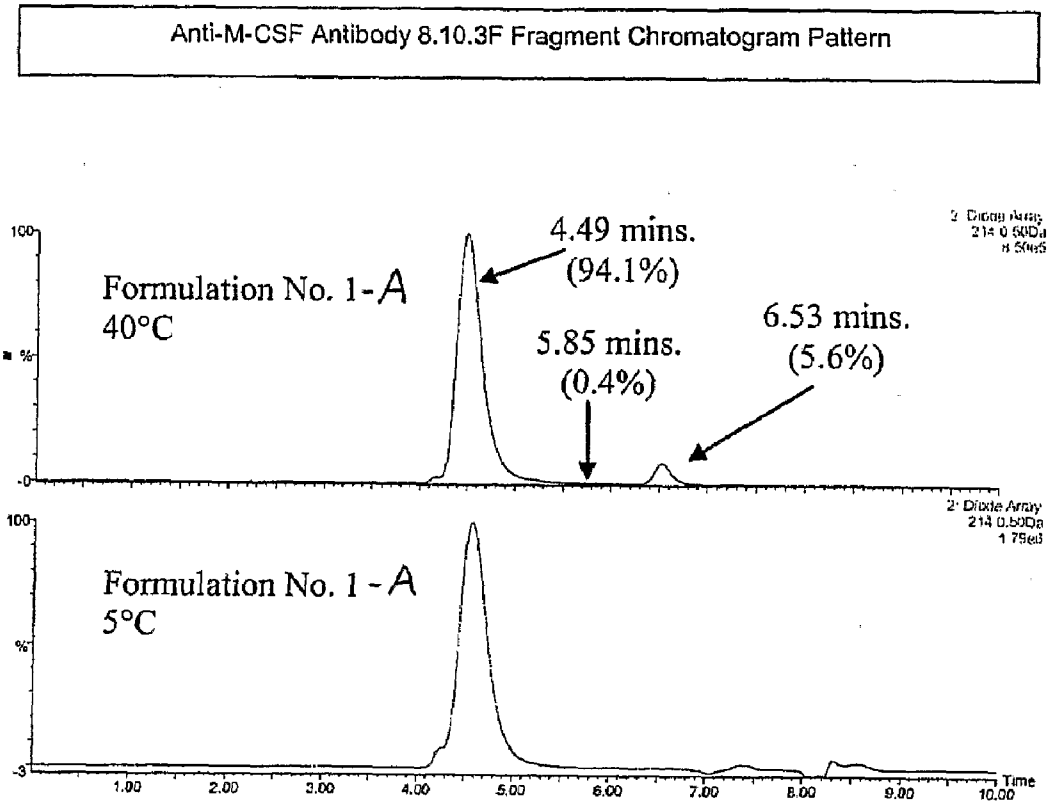

Anti-M-CSF Antibody 8.10.3F Fragment Chromatogram Pattern
Formulation No. 1-A
40°C
4.49 mins.
(94.1%)
5.85 mins.
(0.4%)
6.53 mins.
(5.6%)
Formulation No. 1 - A
5°C
Time
1.00
2.00
3.00
4.00
5.00
6.00
7.00
8.00
9.00
10.00

Figure 11

Anti-M-CSF Antibody 8.10.3F SE-HPLC Chromatogram showing separation of monomer and aggregates

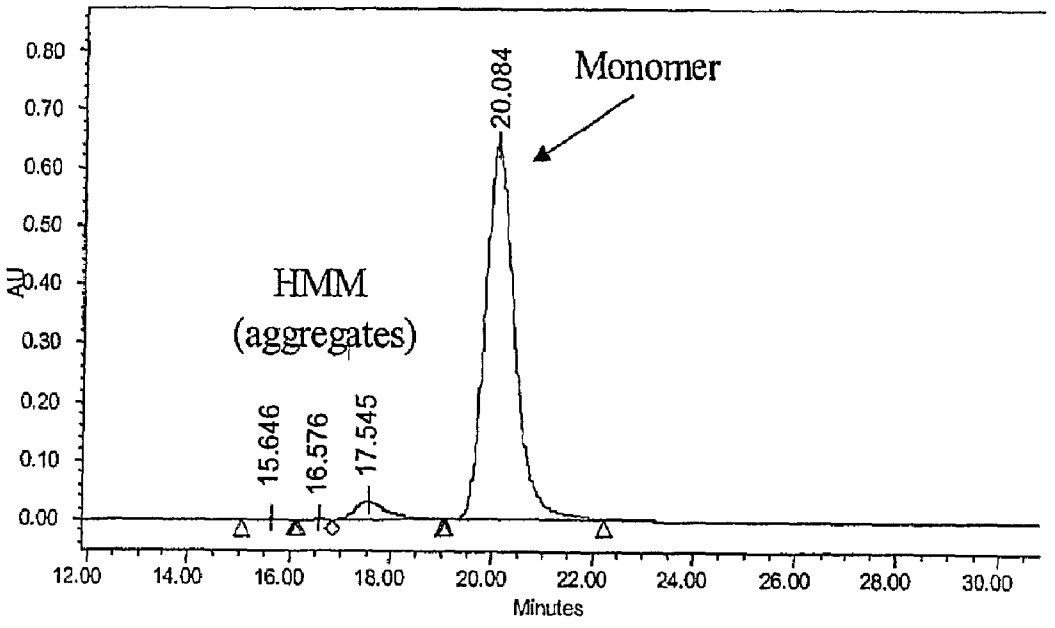


Figure 12

Figure 12A 8.10.3F Heavy Chain [Gamma chain] polynucleotide sequence (SEQ ID NO: 1)

atggagttgggactatgctgggttttccttgttgctattttagaaggtgtccagtgt[GAGGTGCAGCTGGTGGAGTCTG
GGGGAGGCTTGGTACAGCCTGGGGGGTCCCTGAGACTCTCCTGTGCAGCCTCTGGATT
CACCTTCAGTAGTTTTAGTATGACCTGGGTCCGCCAGGCTCCAGGAAAGGGGCTGGAGT
GGGTTTCATACATTAGTAGTAGAAGTAGTACCATATCCTACGCAGACTCTGTGAAGGGCC
GATTCACCATCTCCAGAGACAATGCCAAGAACTCACTGTATCTGCAAATGAACAGCCTGA
GAGACGAGGACACGGCTGTGTATTACTGTGCGAGAGATCCTCTTCTAGCGGGAGCTACC
TTCTTTGACTACTGGGGCCAGGGAACCCTGGTCACCGTCTCCTCAGCC]tccaccaagggccc
atcggtcttccccctggcgccctgctccaggagcacctccgagagcacagcggccctgggctgcctggtcaaggactacttcccc
gaaccggtgacggtgtcgtggaactcaggcgctctgaccagcggcgtgcacaccttcccagctgtcctacagtcctcaggactcta
ctccctcagcagcgtggtgaccgtgccctccagcaacttcggcacccagacctacacctgcaacgtagatcacaagcccagcaa
caccaaggtggacaagacagttgagcgcaaatgttgtgtcgagtgcccaccgtgcccagcaccacctgtggcaggaccgtcagt
cttcctcttccccccaaaacccaaggacaccctcatgatctcccggacccctgaggtcacgtgcgtggtggtggacgtgagccacg
aagaccccgaggtccagttcaactggtacgtggacggcgtggaggtgcataatgccaagacaaagccacgggaggagcagtt
caacagcacgttccgtgtggtcagcgtcctcaccgttgtgcaccaggactggctgaacggcaaggagtacaagtgcaaggtctcc
aacaaaggcctcccagccccatcgagaaaaccatctccaaaaccaaagggcagccccgagaaccacaggtgtacaccctg
cccccatcccgggaggagatgaccaagaaccaggtcagcctgacctgcctggtcaaaggcttctaccccagcgacatcgccgt
ggagtgggagagcaatgggcagccggagaacaactacaagaccacacctcccatgctggactccgacggctccttcttcctcta
cagcaagctcaccgtggacaagagcaggtggcagcaggggaacgtcttctcatgctccgtgatgcatgaggctctgcacaacca
ctacacgcagaagagcctctccctgtctccgggtaaa

Figure 12B 8.10.3F Heavy Chain [Gamma chain] amino acid sequence (SEQ ID NO: 2)

melglcwvflvailegvqc[EVQLVESGGGLVQPGGSLRLSCAASGFTFSSFSMTWVRQAPGKGLE
WVSYISSRSSTISYADSVKGRFTISRDNAKNSLYLQMNSLRDEDTAVYYCARDPLLAGATFF
DYWGQGTLVTVSSA]stkgpsvfplapcsrstsestaalgclvkdyfpepvtvswnsgaltsgvhtfpavlqssglyslssvv
tvpssnfgtqtytcnvdhkpsntkvdktverkccvecppcpappvagpsvflfppkpkdtlmisrtpevtcvvvdvshedpevqfn
wyvdgvevhnaktkpreeqfnstfrvvsvltvvhqdwlngkeykckvsnkglpapiektisktkgqprepqvytlppsreemtknq
vsltclvkgfypsdiavewesngqpennykttppmldsdgsfflyskltvdksrwqqgnvfscsvmhealhnhytqkslslspgk

Key:

Signal peptide: underlined lower case
CDRs 1,2,3: underlined UPPERCASE
Variable domain: UPPER CASE and [bracketed]
Constant domain: lower case
Mutations from germline in bold

Figure 12C 8.10.3F Light Chain [Kappa chain] polynucleotide sequence (SEQ ID NO: 3)

atggaaaccccagcgcagcttctcttcctcctgctactctggctcccagataccaccgga[GAATTTGTGTTGACGCAG
TCTCCAGGCACCCTGTCTTTGTCTCCAGGGGAAAGAGCCACCCTCTCCTGCAGGGCCA
GTCAGAGTGTTAGCAGCAGTTACTTAGCCTGGTACCAGCAGAAACCTGGCCAGGCTCCC
AGGCTCCTCATCTATGGTGCATCCAGCAGGGCCACTGGCATCCCAGACAGGTTCAGTG
GCAGTGGGTCTGGGACAGACTTCACTCTCACCATCAGCAGACTGGAGCCTGAAGATTTT
GCAGTGTATTACTGTCAGCAGTATGGTAGCTCACCTCTCACTTTCGGCGGAGGGACCAA
GGTGGAGATCAAACGA]actgtggctgcaccatctgtcttcatcttcccgccatctgatgagcagttgaaatctggaactgc
ctctgttgtgtgcctgctgaataacttctatcccagagaggccaaagtacagtggaaggtggataacgccctccaatcgggtaactc
ccaggagagtgtcacagagcaggacagcaaggacagcacctacagcctcagcagcaccctgacgctgagcaaagcagact
acgagaaacacaaagtctacgcctgcgaagtcacccatcagggcctgagctcgcccgtcacaaagagcttcaacaggggaga
gtgt

Figure 12D 8.10.3F Light Chain [Kappa chain] amino acid sequence (SEQ ID NO: 4)

metpaqllfllllwlpdttg[EFVLTQSPGTLSLSPGERATLSCRASQSVSSSYLAWYQQKPGQAPRLLI
YGASSRATGIPDRFSGSGSGTDFTLTISRLEPEDFAVYYCQQYGSSPLTFGGGTKVEIKR]tva
apsvfifppsdeqlksgtasvvclinnfypreakvqwkvdnalqsgnsqesvteqdskdstyslsstltlskadyekhkvyacevth
qglsspvtksfnrgec

Key:

Signal peptide: underlined lower case
CDRs 1,2,3: underlined UPPERCASE
Variable domain: UPPER CASE and [bracketed]
Constant domain: lower case
Mutations from germline in **bold**

Figure 13

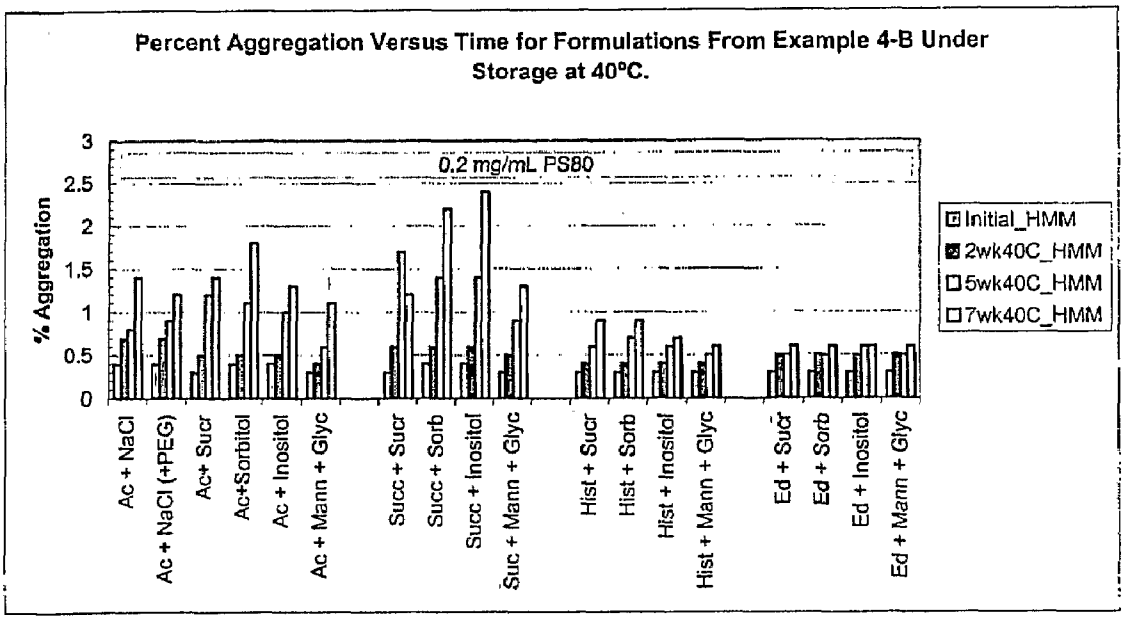

Percent Aggregation Versus Time for Formulations From Example 4-B Under Storage at 40°C.
0.2 mg/mL PS80
% Aggregation
3
2.5
2
1.5
1
0.5
0
Ac + NaCl
Ac + NaCl (+PEG)
Ac+ Sucr
Ac+Sorbitol
Ac + Inositol
Ac + Mann + Glyc
Succ + Sucr
Succ + Sorb
Succ + Inositol
Suc + Mann + Glyc
Hist + Sucr
Hist + Sorb
Hist + Inositol
Hist + Mann + Glyc
Ed + Sucr
Ed + Sorb
Ed + Inositol
Ed + Mann + Glyc
Initial_HMM
2wk40C_HMM
5wk40C_HMM
7wk40C_HMM

Figure 14

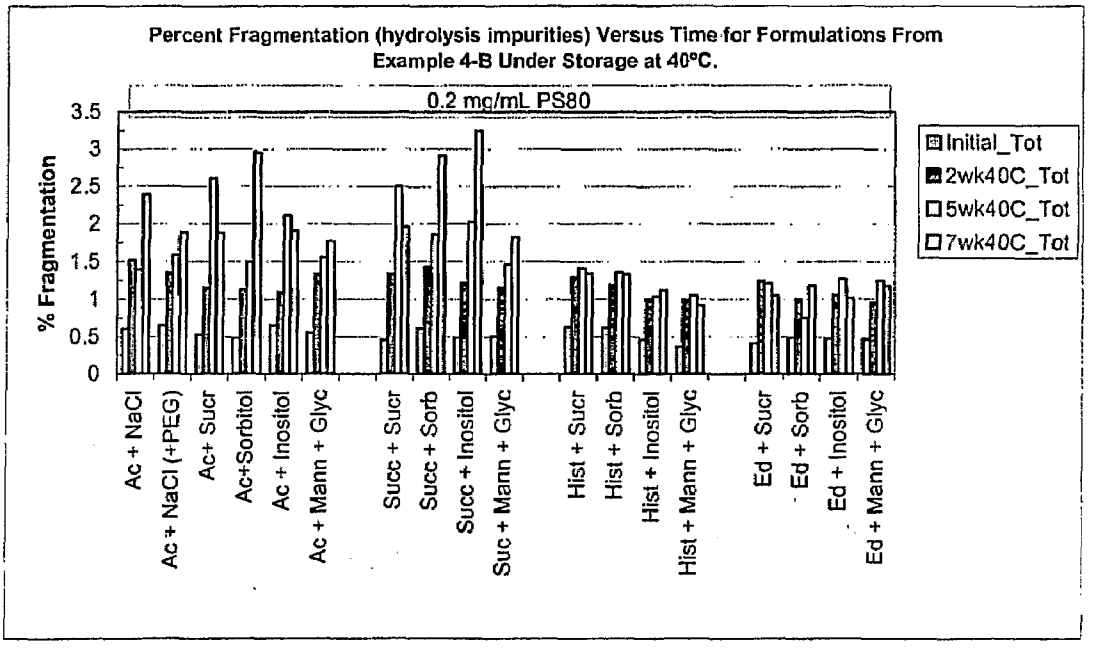

Percent Fragmentation (hydrolysis impurities) Versus Time for Formulations From Example 4-B Under Storage at 40°C.
0.2 mg/mL PS80
% Fragmentation
3.5
3
2.5
2
1.5
1
0.5
0
Ac + NaCl
Ac + NaCl (+PEG)
Ac+ Sucr
Ac+Sorbitol
Ac + Inositol
Ac + Mann + Glyc
Succ + Sucr
Succ + Sorb
Succ + Inositol
Suc + Mann + Glyc
Hist + Sucr
Hist + Sorb
Hist + Inositol
Hist + Mann + Glyc
Ed + Sucr
Ed + Sorb
Ed + Inositol
Ed + Mann + Glyc
Initial_Tot
2wk40C_Tot
5wk40C_Tot
7wk40C_Tot

Figure 15

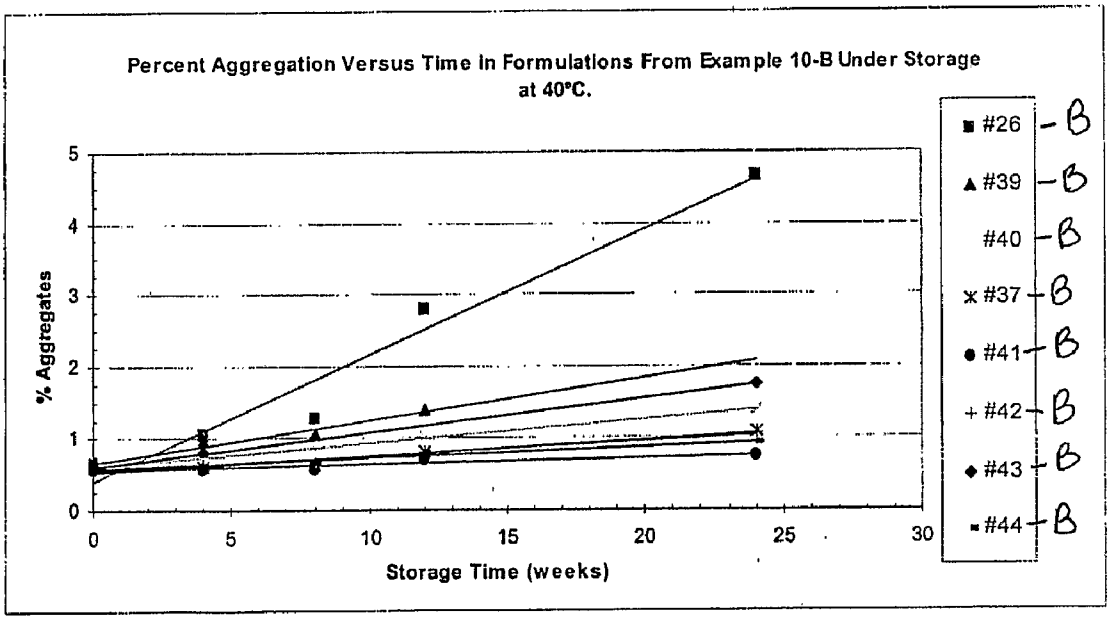

Percent Aggregation Versus Time In Formulations From Example 10-B Under Storage at 40°C.
% Aggregates
Storage Time (weeks)
0
1
2
3
4
5
0
5
10
15
20
25
30
#26 - B
#39 - B
#40 - B
#37 - B
#41 - B
#42 - B
#43 - B
#44 - B

Figure 16

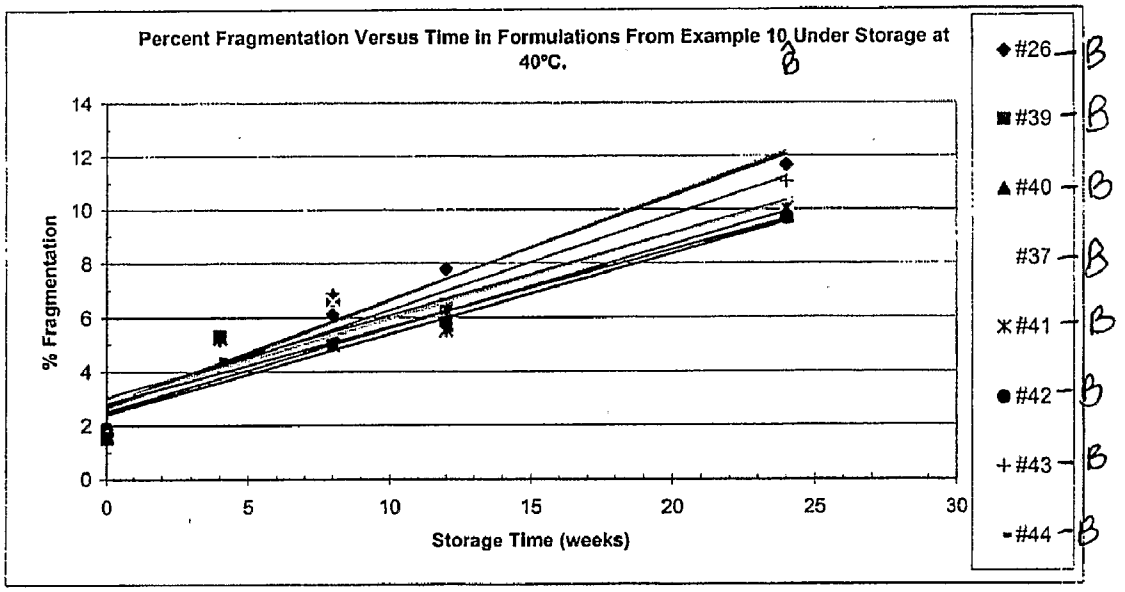

Percent Fragmentation Versus Time in Formulations From Example 10 Under Storage at 40°C.
% Fragmentation
Storage Time (weeks)
0
2
4
6
8
10
12
14
0
5
10
15
20
25
30
#26
#39
#40
#37
#41
#42
#43
#44

Figure 17

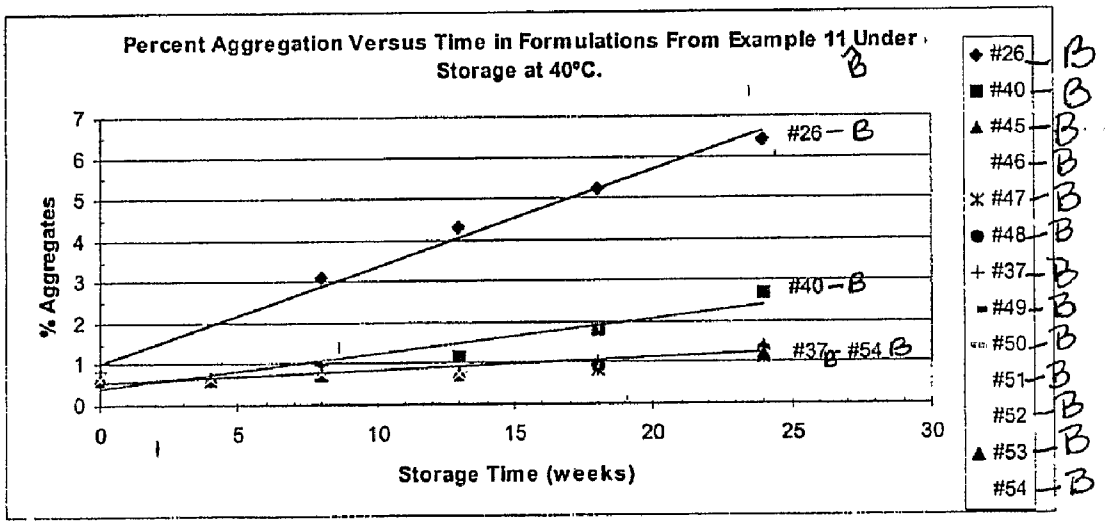

Percent Aggregation Versus Time in Formulations From Example 11 Under Storage at 40°C.
% Aggregates
Storage Time (weeks)
0
1
2
3
4
5
6
7
0
5
10
15
20
25
30
#26
#40
#37
#54
#45
#46
#47
#48
#49
#50
#51
#52
#53

Figure 18

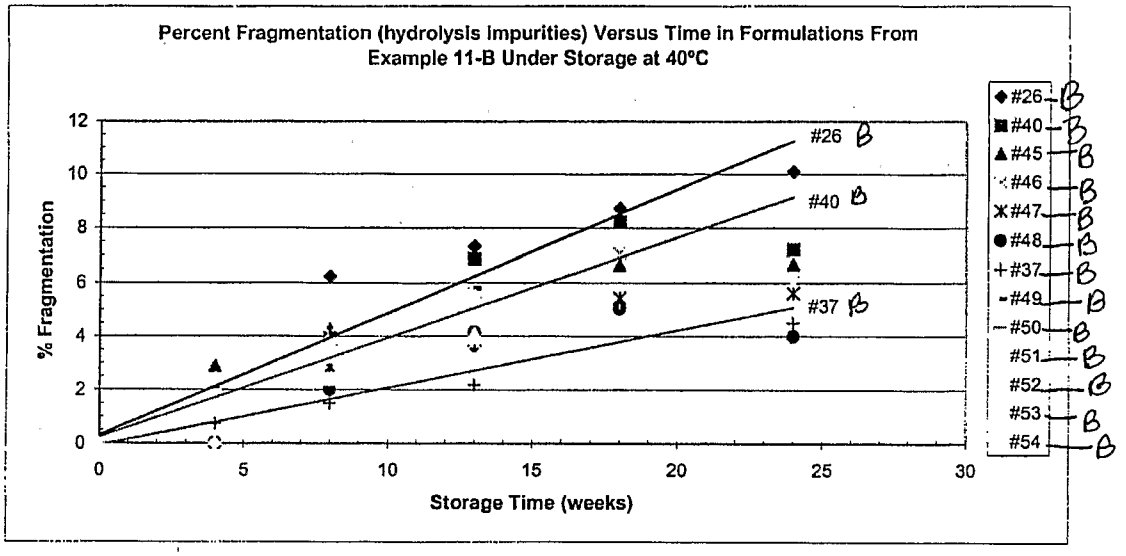

Percent Fragmentation (hydrolysis Impurities) Versus Time in Formulations From Example 11-B Under Storage at 40°C
% Fragmentation
Storage Time (weeks)
0
2
4
6
8
10
12
0
5
10
15
20
25
30
#26 B
#40 B
#37 B
◆#26 B
■#40 B
▲#45 B
#46 B
✳#47 B
●#48 B
+#37 B
-#49 B
—#50 B
#51 B
#52 B
#53 B
#54 B

Figure 19

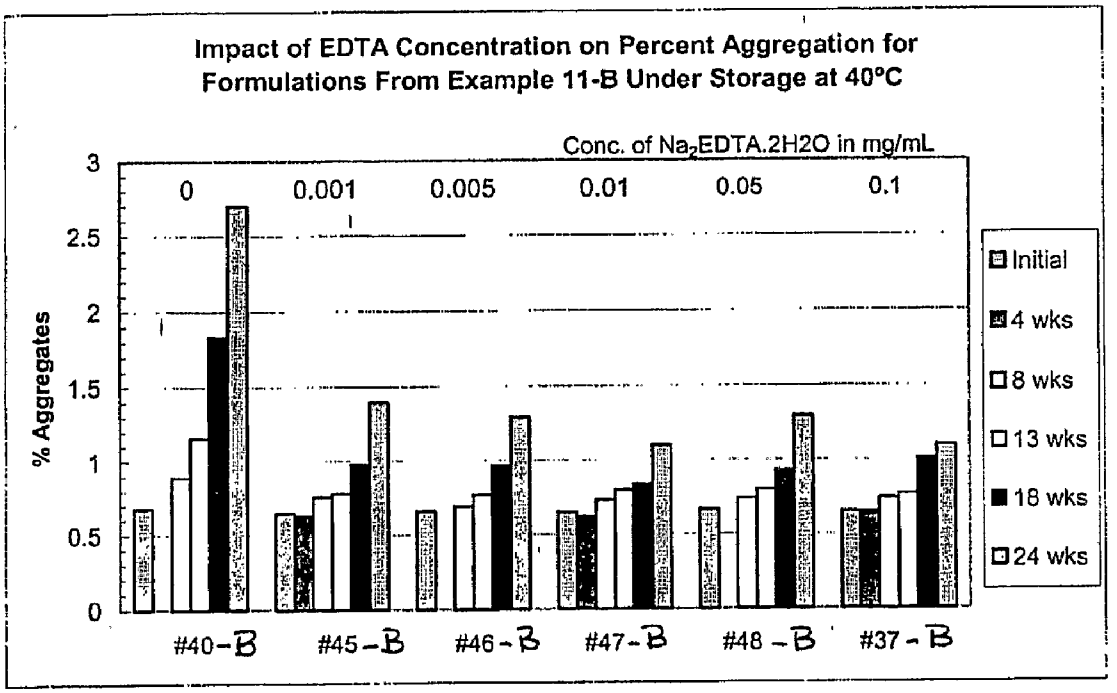

Impact of EDTA Concentration on Percent Aggregation for Formulations From Example 11-B Under Storage at 40°C
Conc. of Na2EDTA.2H2O in mg/mL
0
0.001
0.005
0.01
0.05
0.1
% Aggregates
3
2.5
2
1.5
1
0.5
0
#40-B
#45-B
#46-B
#47-B
#48-B
#37-B
Initial
4 wks
8 wks
13 wks
18 wks
24 wks

Figure 20

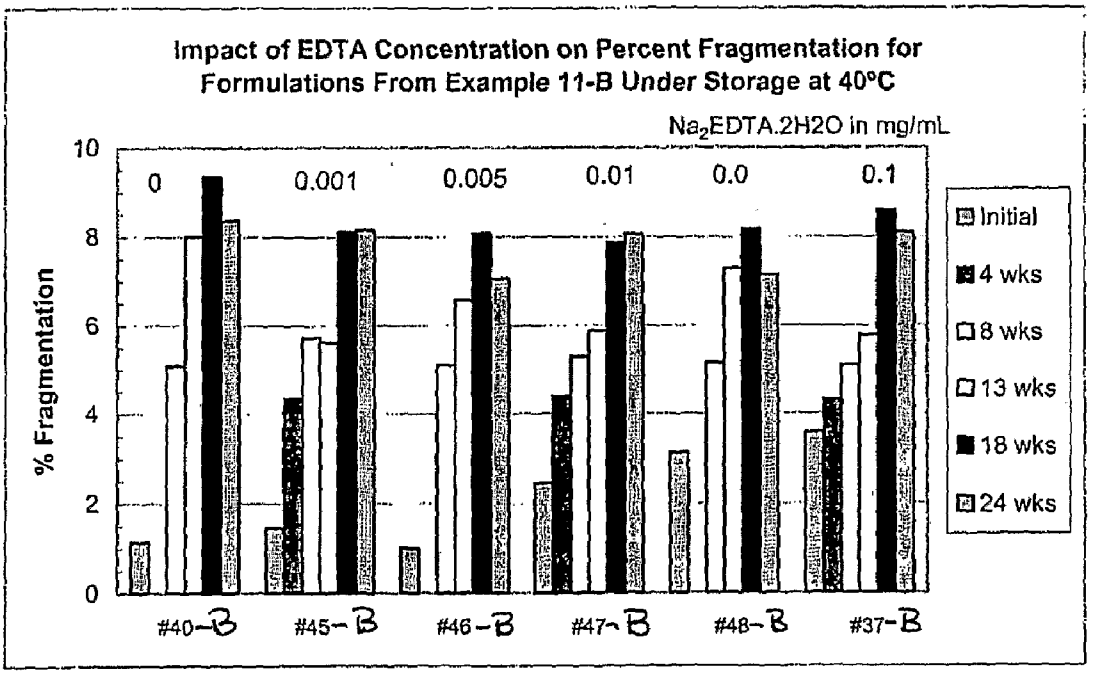

Impact of EDTA Concentration on Percent Fragmentation for Formulations From Example 11-B Under Storage at 40°C
Na2EDTA.2H2O in mg/mL
% Fragmentation
0
2
4
6
8
10
0
0.001
0.005
0.01
0.0
0.1
#40-B
#45-B
#46-B
#47-B
#48-B
#37-B
Initial
4 wks
8 wks
13 wks
18 wks
24 wks

Figure 21

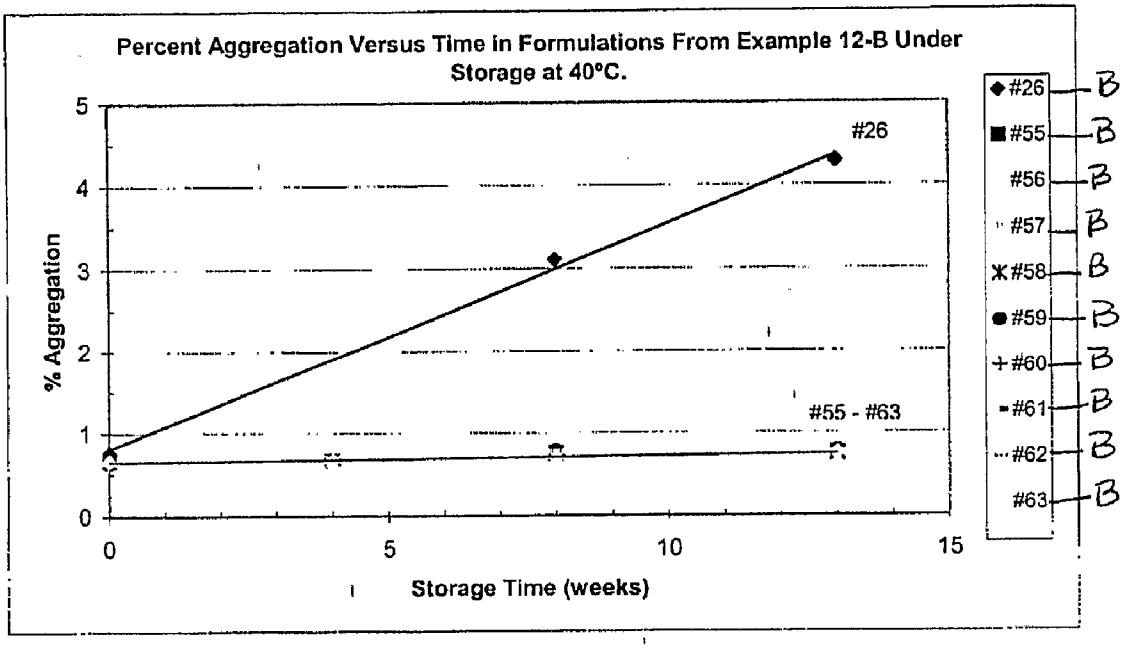

Percent Aggregation Versus Time in Formulations From Example 12-B Under Storage at 40°C.
% Aggregation
Storage Time (weeks)
0
1
2
3
4
5
0
5
10
15
#26
#55 - #63
#26
#55
#56
#57
#58
#59
#60
#61
#62
#63
B

Figure 22

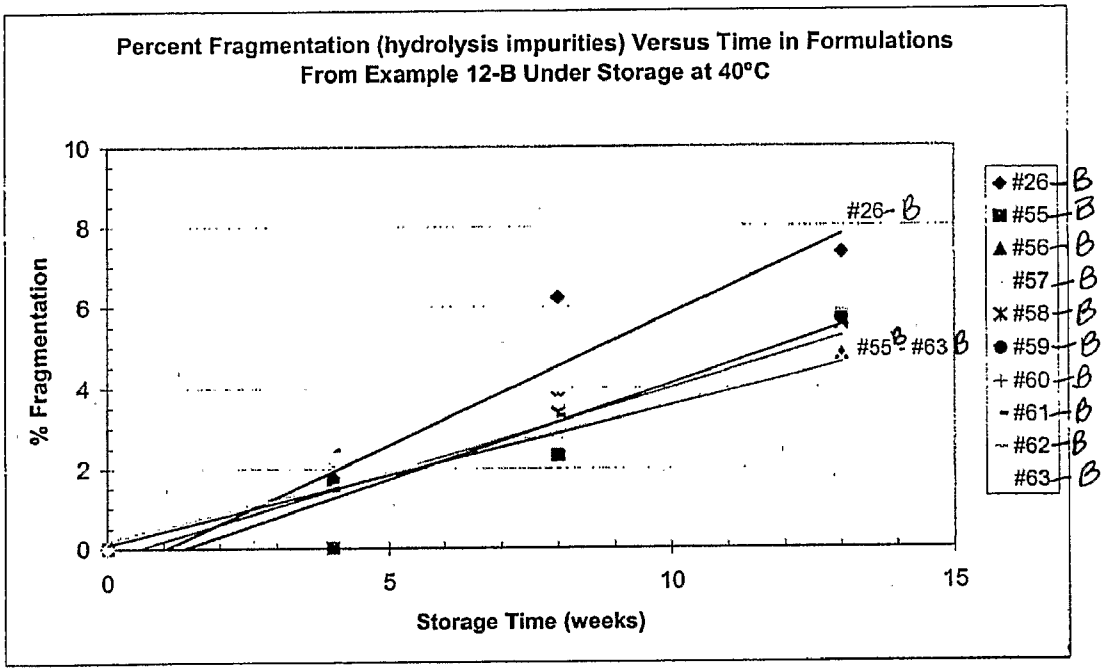

Percent Fragmentation (hydrolysis impurities) Versus Time in Formulations From Example 12-B Under Storage at 40°C
% Fragmentation
Storage Time (weeks)
0
2
4
6
8
10
0
5
10
15
#26- B
#55 B #63 B
◆ #26 B
■ #55 B
▲ #56 B
#57 B
✱ #58 B
● #59 B
+ #60 B
- #61 B
~ #62 B
#63 B

Figure 23

Figure 23A Ticilimumab (11.2.1) Heavy Chain DNA (SEQ ID NO: 21)

```
atggagtttg ggctgagctg ggttttcctc gttgctcttt taagaggtgt ccagtgtcag   60
gtgcagctgg tggagtctgg gggaggcgtg gtccagcctg ggaggtccct gagactctcc  120
tgtgcagcgt ctggattcac cttcagtagc tatggcatgc actgggtccg ccaggctcca  180
ggcaaggggc tggagtgggt ggcagttata tggtatgatg gaagtaataa atactatgca  240
gactccgtga agggccgatt caccatctcc agagacaatt ccaagaacac gctgtatctg  300
caaatgaaca gcctgagagc cgaggacacg gctgtgtatt actgtgcgag agatccgagg  360
ggagctaccc tttactacta ctactacggt atggacgtct ggggccaagg gaccacggtc  420
accgtctcct cagcctccac caagggccca tcggtcttcc ccctggcgcc ctgctccagg  480
agcacctccg agagcacagc ggccctgggc tgcctggtca aggactactt ccccgaaccg  540
gtgacggtgt cgtggaactc aggcgctctg accagcggcg tgcacacctt cccagctgtc  600
ctacagtcct caggactcta ctccctcagc agcgtggtga ccgtgccctc cagcaacttc  660
ggcacccaga cctacacctg caacgtagat cacaagccca gcaacaccaa ggtggacaag  720
acagttgagc gcaaatgttg tgtcgagtgc ccaccgtgcc cagcaccacc tgtggcagga  780
ccgtcagtct tcctcttccc cccaaaaccc aaggacaccc tcatgatctc ccggaccccct  840
gaggtcacgt gcgtggtggt ggacgtgagc cacgaagacc ccgaggtcca gttcaactgg  900
tacgtggacg gcgtggaggt gcataatgcc aagacaaagc cacgggagga gcagttcaac  960
agcacgttcc gtgtggtcag cgtcctcacc gttgtgcacc aggactggct gaacggcaag 1020
gagtacaagt gcaaggtctc caacaaaggc ctcccagccc ccatcgagaa aaccatctcc 1080
aaaaccaaag ggcagccccg agaaccacag gtgtacaccc tgcccccatc ccgggaggag 1140
atgaccaaga accaggtcag cctgacctgc ctggtcaaag gcttctaccc cagcgacatc 1200
gccgtggagt gggagagcaa tgggcagccg gagaacaact acaagaccac acctcccatg 1260
ctggactccg acggctcctt cttcctctac agcaagctca ccgtggacaa gagcaggtgg 1320
cagcagggga acgtcttctc atgctccgtg atgcatgagg ctctgcacaa ccactacacg 1380
cagaagagcc tctccctgtc tccgggtaaa tga                               1413
```

Figure 23B Ticilimumab (11.2.1) Heavy Chain Amino Acid (SEQ ID NO: 22)

```
IQVQLVESGGG VVQPGRSLRL SCAASGFTFS SYGMHWVRQA PGKGLEWVAV IWYDGSNKYY   60
ADSVKGRFTI  SRDNSKNTLY LQMNSLRAED TAVYYCARDP RGATLYYYYY GMDVWGQGTT  120
VTVSSJASTKG PSVFPLAPCS RSTSESTAAL GCLVKDYFPE PVTVSWNSGA LTSGVHTFPA  180
VLQSSGLYSL  SSVVTVPSSN FGTQTYTCNV DHKPSNTKVD KTVERKCCVE CPPCPAPPVA  240
GPSVFLFPPK  PKDTLMISRT PEVTCVVVDV SHEDPEVQFN WYVDGVEVHN AKTKPREEQF  300
NSTFRVVSVL  TVVHQDWLNG KEYKCKVSNK GLPAPIEKTI SKTKGQPREP QVYTLPPSRE  360
EMTKNQVSLT  CLVKGFYPSD IAVEWESNGQ PENNYKTTPP MLDSDGSFFL YSKLTVDKSR  420
WQQGNVFSCS  VMHEALHNHY TQKSLSLSPG K                                  451
```

The variable region (SEQ ID NO: 25) is depicted [between brackets] and the CDRs are underlined. CDR1 is indicated by SEQ ID NO: 27, CDR2 by SEQ ID NO: 28, and CDR3 by SEQ ID NO: 29.

Figure 23C Ticilimumab (11.2.1) Light Chain DNA (SEQ ID NO: 23)

```
atggacatga gggtccccgc tcagctcctg gggctcctgc tactctggct ccgaggtgcc   60
agatgtgaca tccagatgac ccagtctcca tcctccctgt ctgcatctgt aggagacaga   120
gtcaccatca cttgccgggc aagtcagagc attaacagct atttagattg gtatcagcag   180
aaaccaggga aagcccctaa actcctgatc tatgctgcat ccagtttgca aagtggggtc   240
ccatcaaggt tcagtggcag tggatctggg acagatttca ctctcaccat cagcagtctg   300
caacctgaag attttgcaac ttactactgt caacagtatt acagtactcc attcactttc   360
ggccctggga ccaaagtgga aatcaaacga actgtggctg caccatctgt cttcatcttc   420
ccgccatctg atgagcagtt gaaatctgga actgcctctg ttgtgtgcct gctgaataac   480
ttctatccca gagaggccaa agtacagtgg aaggtggata acgccctcca atcgggtaac   540
tcccaggaga gtgtcacaga gcaggacagc aaggacagca cctacagcct cagcagcacc   600
ctgacgctga gcaaagcaga ctacgagaaa cacaaagtct acgcctgcga agtcacccat   660
cagggcctga gctcgcccgt cacaaagagc ttcaacaggg gagagtgtta gtga          714
```

Figure 23D Ticilimumab (11.2.1) Light Chain Amino Acid (SEQ ID NO: 24)

```
[DIQMTQSPSS LSASVGDRVT ITCRASQSIN SYLDWYQQKP GKAPKLLIYA ASSLQSGVPS   60
RFSGSGSGTD FTLTISSLQP EDFATYYCQQ YYSTPFTFGP GTKVEIK]RTV AAPSVFIFPP  120
SDEQLKSGTA SVVCLLNNFY PREAKVQWKV DNALQSGNSQ ESVTEQDSKD STYSLSSTLT  180
LSKADYEKHK VYACEVTHQG LSSPVTKSFN RGEC                               214
```

The variable region (SEQ ID NO: 26) is depicted [between brackets] and the CDRs are underlined. CDR1 is indicated by SEQ ID NO: 30, CDR2 by SEQ ID NO: 31, and CDR3 by SEQ ID NO: 32.

Figure 24

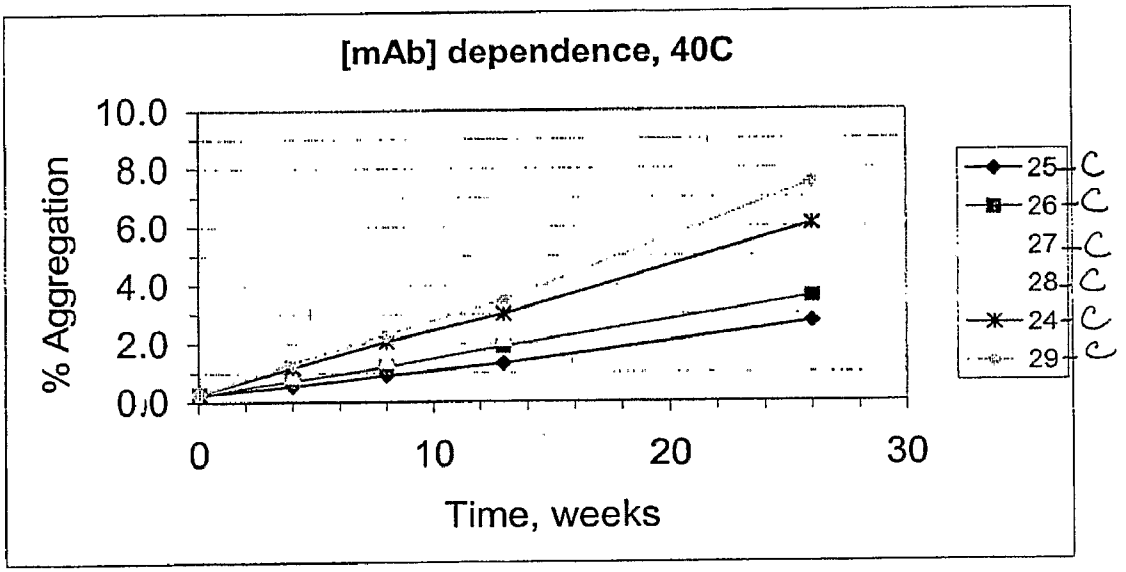

[mAb] dependence, 40C
% Aggregation
10.0
8.0
6.0
4.0
2.0
0.0
0
10
20
30
Time, weeks
25 C
26 C
27 C
28 C
24 C
29 C

Figure 25

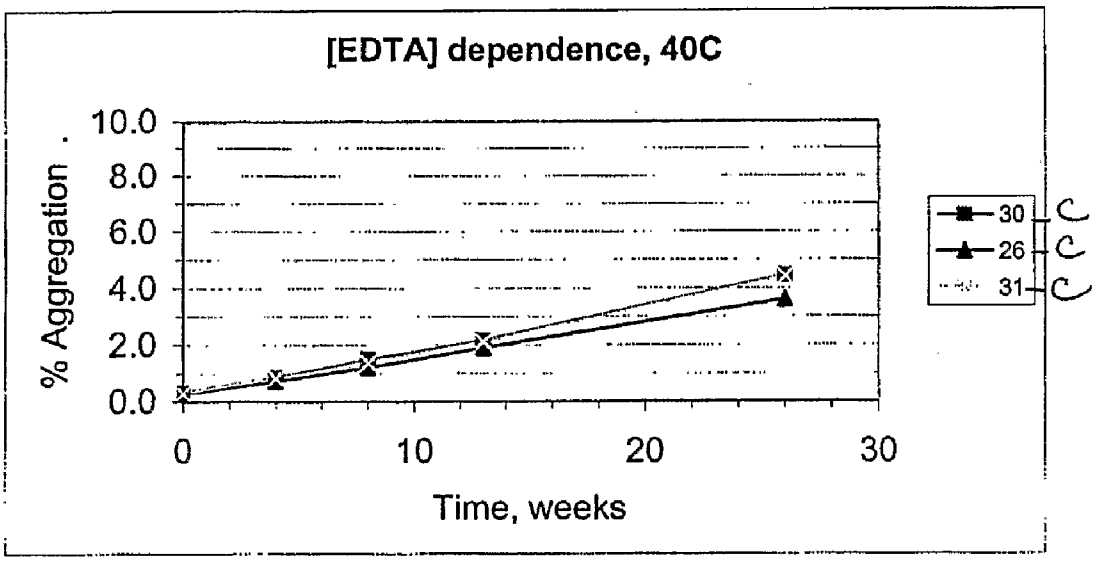

[EDTA] dependence, 40C
% Aggregation .
10.0
8.0
6.0
4.0
2.0
0.0
0
10
20
30
Time, weeks
30 C
26 C
31 C

Figure 26

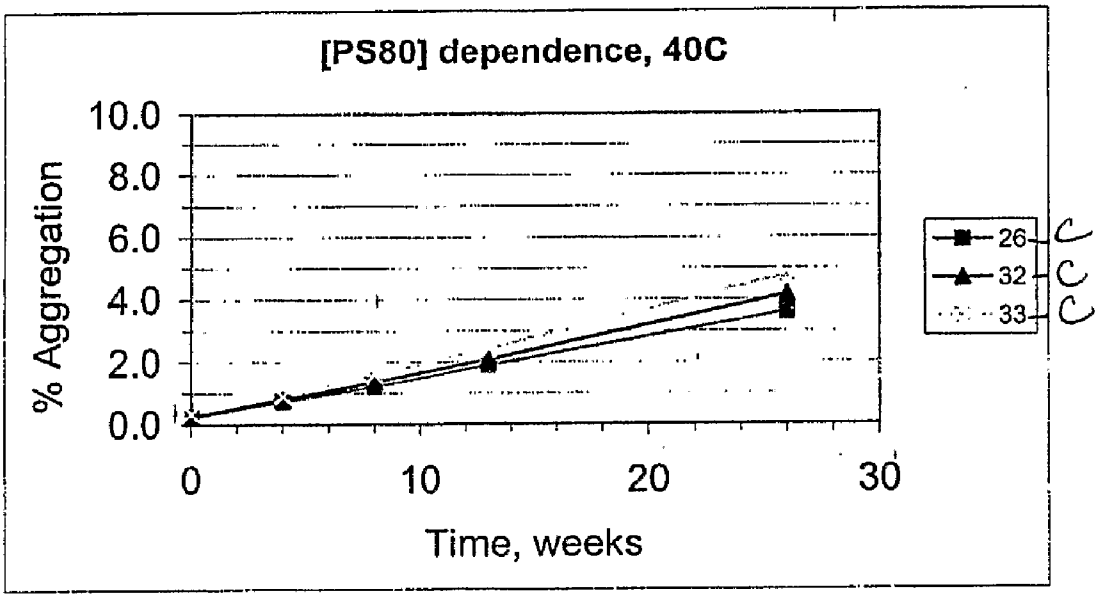

[PS80] dependence, 40C
% Aggregation
10.0
8.0
6.0
4.0
2.0
0.0
0
10
20
30
Time, weeks
26
32
33

Figure 27

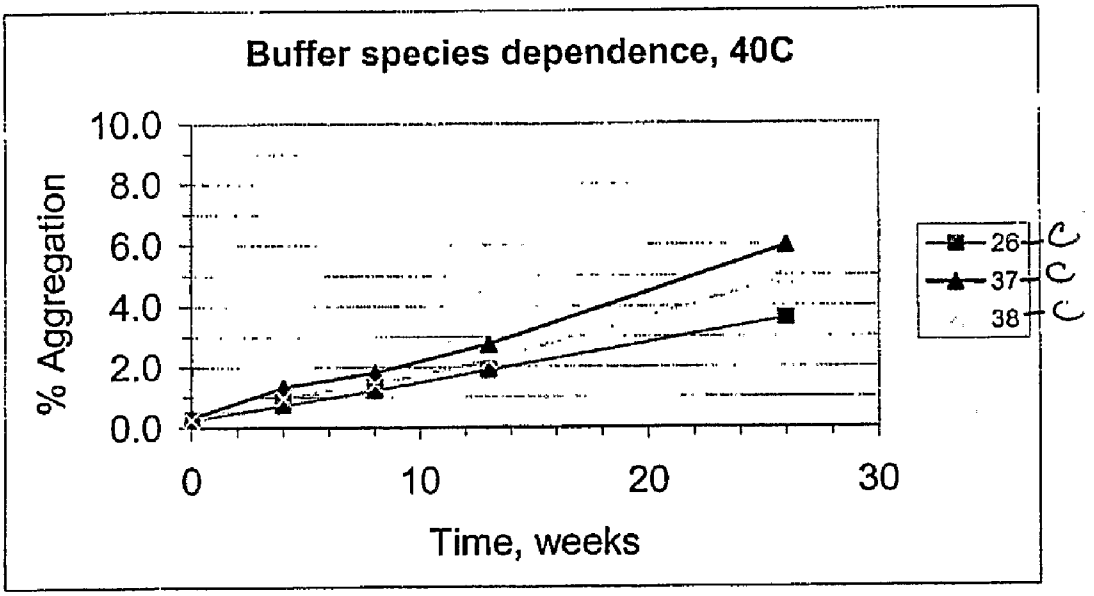

Buffer species dependence, 40C
% Aggregation
10.0
8.0
6.0
4.0
2.0
0.0
0
10
20
30
Time, weeks
26 C
37 C
38 C

Figure 28

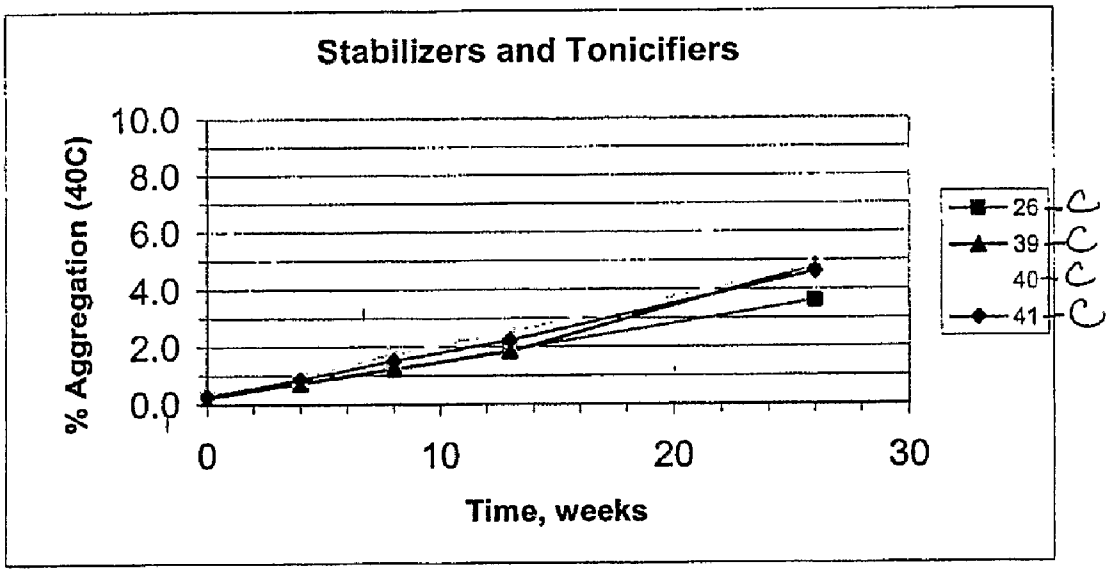

Stabilizers and Tonicifiers
% Aggregation (40C)
10.0
8.0
6.0
4.0
2.0
0.0
0
10
20
30
Time, weeks
26 C
39 C
40 C
41 C

Figure 29

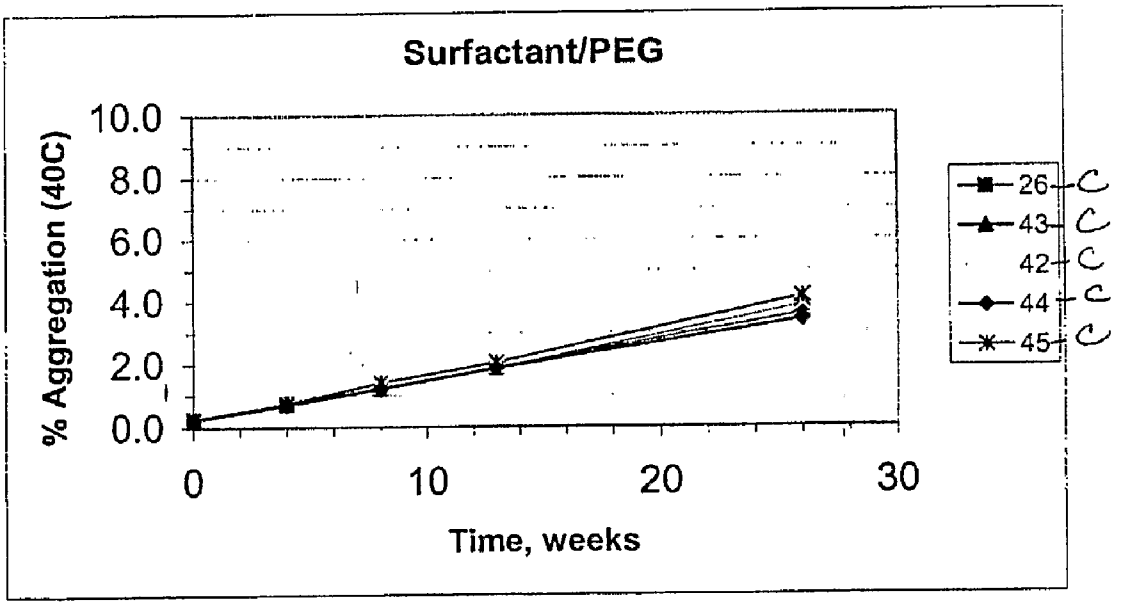

Surfactant/PEG
% Aggregation (40C)
10.0
8.0
6.0
4.0
2.0
0.0
0
10
20
30
Time, weeks
26
43
42
44
45

Figure 30

Figure 30A 7.16.6 Heavy Chain Nucleotide Sequence (SEQ ID NO: 41)

```
   1  atggactgga cctggagcat ccttttcttg gtggcagcag caacaggtgc
  51  ccactccCAG GTTCAGCTGG TGCAGTCTGG AGCTGAGGTG AAGAAGCCTG
 101  GGGCCTCAGT GAAGGTCTCC TGCAAGGCTT CTGGTTACAC CTTTACCAGC
 151  TATGGTATCA ACTGGGTGCG ACAGGCCCCT GGACAAGGGC TTGAGTGGAT
 201  GGGATGGATC AGCGTTTACA GTGGTAACAC AAACTATGCA CAGAAGGTCC
 251  AGGGCAGAGT CACCATGACC GCAGACACAT CCACGAGCAC AGCCTACATG
 301  GACCTGAGGA GCCTGAGATC TGACGACACG GCCGTGTATT ACTGTGCGAG
 351  AGAGGGTAGC AGCTCGTCCG GAGACTACTA TTACGGTATG GACGTCTGGG
 401  GCCAAGGGAC CACGGTCACC GTCTCCTCAG CCTCCACCAA GGGCCCATCG
 451  GTCTTCCCCC TGGCGCCCTG CTCCAGGAGC ACCTCCGAGA GCACAGCGGC
 501  CCTGGGCTGC CTGGTCAAGG ACTACTTCCC CGAACCGGTG ACGGTGTCGT
 551  GGAACTCAGG CGCTCTGACC AGCGGCGTGC ACACCTTCCC AGCTGTCCTA
 601  CAGTCCTCAG GACTCTACTC CCTCAGCAGC GTGGTGACCG TGCCCTCCAG
 651  CAACTTCGGC ACCCAGACCT ACACCTGCAA CGTAGATCAC AAGCCCAGCA
 701  ACACCAAGGT GGACAAGACA GTTGAGCGCA AATGTTGTGT CGAGTGCCCA
 751  CCGTGCCCAG CACCACCTGT GGCAGGACCG TCAGTCTTCC TCTTCCCCCC
 801  AAAACCCAAG GACACCCTCA TGATCTCCCG GACCCCTGAG GTCACGTGCG
 851  TGGTGGTGGA CGTGAGCCAC GAAGACCCCG AGGTCCAGTT CAACTGGTAC
 901  GTGGACGGCG TGGAGGTGCA TAATGCCAAG ACAAAGCCAC GGGAGGAGCA
 951  GTTCAACAGC ACGTTCCGTG TGGTCAGCGT CCTCACCGTT GTGCACCAGG
1001  ACTGGCTGAA CGGCAAGGAG TACAAGTGCA AGGTCTCCAA CAAAGGCCTC
1051  CCAGCCCCCA TCGAGAAAAC CATCTCCAAA ACCAAAGGGC AGCCCCGAGA
1101  ACCACAGGTG TACACCCTGC CCCCATCCCG GGAGGAGATG ACCAAGAACC
1151  AGGTCAGCCT GACCTGCCTG GTCAAAGGCT TCTACCCCAG CGACATCGCC
1201  GTGGAGTGGG AGAGCAATGG GCAGCCGGAG AACAACTACA AGACCACACC
1251  TCCCATGCTG GACTCCGACG GCTCCTTCTT CCTCTACAGC AAGCTCACCG
1301  TGGACAAGAG CAGGTGGCAG CAGGGGAACG TCTTCTCATG CTCCGTGATG
1351  CATGAGGCTC TGCACAACCA CTACACGCAG AAGAGCCTCT CCCTGTCTCC
1401  GGGTAAATGA
```

Figure 30B 7.16.6 Heavy Chain Protein Sequence (SEQ ID NO: 42)

```
   1  mdwtwsilfl vaaatgahsQ VQLVQSGAEV KKPGASVKVS CKASGYTFTS
  51  YGINWVRQAP GQGLEWMGWI SVYSGNTNYA QKVQGRVTMT ADTSTSTAYM
 101  DLRSLRSDDT AVYYCAREGS SSSGDYYYGM DVWGQGTTVT VSSASTKGPS
 151  VFPLAPCSRS TSESTAALGC LVKDYFPEPV TVSWNSGALT SGVHTFPAVL
 201  QSSGLYSLSS VVTVPSSNFG TQTYTCNVDH KPSNTKVDKT VERKCCVECP
 251  PCPAPPVAGP SVFLFPPKPK DTLMISRTPE VTCVVVDVSH EDPEVQFNWY
 301  VDGVEVHNAK TKPREEQFNS TFRVVSVLTV VHQDWLNGKE YKCKVSNKGL
 351  PAPIEKTISK TKGQPREPQV YTLPPSREEM TKNQVSLTCL VKGFYPSDIA
 401  VEWESNGQPE NNYKTTPPML DSDGSFFLYS KLTVDKSRWQ QGNVFSCSVM
 451  HEALHNHYTQ KSLSLSPGK
```

Figure 30C 7.16.6 Light Chain Nucleotide Sequence SEQ ID NO: 43

```
  1   atgaggctcc ctgctcagct cctggggctg ctaatgctct ggatacctgg
 51   atccagtgca GATATTGTGA TGACCCAGAC TCCACTCTCT CTGTCCGTCA
101   CCCCTGGACA GCCGGCCTCC ATCTCCTGCA AGTCTAGTCA GAGCCTCCTG
151   CATACTGATG GAACGACCTA TTTGTATTGG TACCTGCAGA AGCCAGGCCA
201   GCCTCCACAG CTCCTGATCT ATGAAGTTTC CAACCGGTTC TCTGGAGTGC
251   CAGATAGGTT CAGTGGCAGC GGGTCAGGGA CAGATTTCAC ACTGAAAATC
301   AGCCGGGTGG AGGCTGAGGA TGTTGGGATT TATTACTGCA TGCAAAATAT
351   ACAGCTTCCG TGGACGTTCG GCCAAGGGAC CAAGGTGGAA ATCAAACGAA
401   CTGTGGCTGC ACCATCTGTC TTCATCTTCC CGCCATCTGA TGAGCAGTTG
451   AAATCTGGAA CTGCCTCTGT TGTGTGCCTG CTGAATAACT TCTATCCCAG
501   AGAGGCCAAA GTACAGTGGA AGGTGGATAA CGCCCTCCAA TCGGGTAACT
551   CCCAGGAGAG TGTCACAGAG CAGGACAGCA AGGACAGCAC CTACAGCCTC
601   AGCAGCACCC TGACGCTGAG CAAAGCAGAC TACGAGAAAC ACAAAGTCTA
651   CGCCTGCGAA GTCACCCATC AGGGCCTGAG CTCGCCCGTC ACAAAGAGCT
701   TCAACAGGGG AGAGTGTTAG TGA
```

Figure 30D 7.16.6 Light Chain Protein Sequence (SEQ ID NO: 44)

```
  1   mrlpaqllgl lmlwipgssa DIVMTQTPLS LSVTPGQPAS ISCKSSQSLL
 51   HTDGTTYLYW YLQKPGQPPQ LLIYEVSNRF SGVPDRFSGS GSGTDFTLKI
101   SRVEAEDVGI YYCMQNIQLP WTFGQGTKVE IKRTVAAPSV FIFPPSDEQL
151   KSGTASVVCL LNNFYPREAK VQWKVDNALQ SGNSQESVTE QDSKDSTYSL
201   SSTLTLSKAD YEKHKVYACE VTHQGLSSPV TKSFNRGEC
```

Figure 30E MAdCAM-IgG$_1$ Fc Fusion Protein: (SEQ ID NO: 45)

MDFGLALLLAGLLGLLLGQSLQVKPLQVEPPEPVVAVALGASRQLTCRLACADRGASVQWR
GLDTSLGAVQSDTGRSVLTVRNASLSAAGTRVCVGSCGGRTFQHTVQLLVYAFPDQLTVSP
AALVPGDPEVACTAHKVTPVDPNALSFSLLVGGQELEGAQALGPEVQEEEEEPQGDEDVLF
RVTERWRLPPLGTPVPPALYCQATMRLPGLELSHRQAIPVLHSPTSPEPPDTTSPESPDTTS
PESPDTTSQEPPDTTSQEPPDTTSQEPPDTTSPEPPDKTSPEPAPQQGSTHTPRSPGSTRT
RRPEIQPKSCDKTHTCPPCPAPELLGGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSHEDPE
VKFNWYVDGVEVHNAKTKPREEQYNSTYRVVSVLTVLHQDWLNGKEYKCKVSNKALPAPIE
KTISKAKGQPREPQVYTLPPSRDELTKNQVSLTCLVKGFYPSDIAVEWESNGQPENNYKATP
PVLDSDGSFFLYSKLTVDKSRWQQGNVFSCSVMHEALHNHYTQKSLSLSPGK

PLATFORM ANTIBODY COMPOSITIONS

CROSS-REFERENCE TO RELATED PATENTS AND PATENT APPLICATIONS

[0001] This application claims the benefit of U.S. Provisional Patent Application Ser. No. 60/659,766 filed Mar. 8, 2005; U.S. Provisional Patent Application Ser. No. 60/728,165 filed Oct. 19, 2005; U.S. Provisional Patent Application Ser. No. 60/752,712 filed Dec. 20, 2005; and U.S. Provisional Patent Application Ser. No. 60/762,456 filed Jan. 26, 2006, all of which are incorporated by reference herein in their entireties.

BACKGROUND OF THE INVENTION

[0002] In recent years, advances in biotechnology have made it possible to produce a variety of antibody drugs for therapeutic applications. Several antibody drugs have been developed for use in the diagnosis, prevention, and treatment of many different diseases and disorders. However, because antibodies are larger and more complex than traditional inorganic drugs (i.e., possessing multiple functional groups in addition to complex three-dimensional structures), the composition of such antibodies poses special problems. For example, in order to successfully market an antibody, the product should have adequate stability over time. Developing stable antibody dosage forms presents significant challenges because, like other protein compositions, antibody compositions are subject to the same concerns regarding physical and chemical degradation of the antibody in the composition over time.

[0003] Physical degradations are changes in higher order protein structures (secondary, tertiary and quaternary) and do not involve covalent modification of the protein. Examples of physical degradations include aggregation, fragmentation, adsorption, denaturation, and precipitation. In contrast, chemical degradations involve modification of the primary structure of proteins via bond formation or cleavage, thereby yielding a new chemical entity. Examples of chemical degradations include deamidation, racemization, isomerization, beta elimination, disulfide exchange and hydrolysis. While technically distinct, physical and chemical degradations are often interrelated. For example, a partially unfolded protein (physically degraded) can result in an increase in oxidation (chemical degradation). In general, antibody compositions should exhibit acceptable chemical and physical stability under the expected range of storage and use conditions, i.e., the antibody composition should have a sufficient shelf life yet remain biologically active.

[0004] Many antibody preparations intended for human use require various stabilizers to prevent denaturation, aggregation and other alterations to the proteins prior to the use of the preparation. This instability is often increased when the antibody preparation is stored over time and during shipping.

[0005] For example, U.S. Published Application No. 20050059113 reports a composition containing a human IgG2 anti-M-CSF antibody, 20 mM sodium acetate, and 140 mM sodium chloride, at pH 5.5.

[0006] U.S. Pat. No. 6,682,736 reports a composition containing a human IgG2 anti-CTLA-4 antibody in phosphate buffered saline.

[0007] International Patent Application Number PCT/US2005/000370 reports a composition containing a human IgG2 anti-MAdCAM antibody, 20 mM sodium acetate, 0.2 mg/ml polysorbate 80, and 140 mM sodium chloride, at pH 5.5.

[0008] WO 2004007520 reports that proteins susceptible to oxidation can be formulated with selected metal chelators such as DTPA, EGTA and/or DEF to protect the protein. WO 2004007520 notes, however, that the addition of the metal chelator EDTA actually increased oxidation damage to and aggregation of formulated proteins.

[0009] WO 2003039485 reports an aqueous composition containing 100 mg/ml of a humanized IgG1 anti-IL-2 antibody, 50 mM histidine, 115 mM sodium chloride, 0.03% Tween®-80, and 0.05% EDTA, at pH 6.0.

[0010] WO 97/45140 reports an aqueous composition containing 100 mg/ml of an anti-CD4 antibody, 100 mM sodium citrate, and 0.05 mM EDTA, at pH 6.0.

[0011] U.S. Published Application No. 20040191243 reports a liquid composition containing 100 mg/mL of a human IgG2 anti-IL8 antibody, 40 mM histidine, 40 mM arginine, 150 mM sucrose, and 0.04% polysorbate 20.

[0012] U.S. Pat. No. 6,267,958 reports a reconstituted composition containing 100 mg/ml of a humanized IgG1 antibody, 20 mM histidine, 340 mM sucrose, 0.04% polysorbate 20, and 0.9% benzyl alcohol, at pH 6.0.

[0013] U.S. Pat. No. 6,171,586 reports an aqueous antibody composition containing 25 mg/ml of a humanized IgG1 anti-CD20 antibody, 25 mM acetate, 150 mM trehalose, 0.9% benzyl alcohol, and 0.02% polysorbate 20, at pH 5.

[0014] U.S. Pat. No. 5,654,403 reports a liquid composition containing a humanized IgG1 anti-CD52 antibody, phosphate buffered saline, and between 0.05 mM and 5 mM of a chelator of copper ions.

[0015] Therefore, a major aim in the development of an antibody drug composition is to maintain protein stability, solubility, and bioactivity. Given the time and resources necessary to produce an antibody product, compositions that reduce product loss are desirable. Accordingly, the present application discloses novel antibody compositions that exhibit improved chemical and/or physical stability relative to antibody compositions previously disclosed in the literature.

SUMMARY

[0016] In one aspect, the present invention provides a composition comprising at least one human IgG_2 antibody; and a chelating agent.

[0017] The present invention also provides a composition comprising at least one monoclonal human IgG2 antibody and a chelating agent, wherein the composition comprises an amount of the chelating agent sufficient to stabilize the composition when maintained at a temperature of about 40° C. for a period of at least about 26 weeks.

[0018] The present invention also provides a liquid pharmaceutical composition comprising at least one human IgG2 monoclonal antibody and a pharmaceutically acceptable chelating agent, wherein the molar concentration of the antibody ranges from about 0.0006 millimolar to about 1.35 millimolar and the molar concentration of the chelating agent ranges from about 0.003 millimolar to about 50 millimolar, and wherein the molar ratio of antibodies to chelating agent ranges from about 0.00001 to about 450.

[0019] The present invention also provides a process for preparing a liquid human IgG2 pharmaceutical composition

comprising mixing at least one human IgG2 antibody in solution; with at least one chelating agent.

[0020] The present invention also provides a stable composition comprising at least one monoclonal human IgG2 antibody and a chelating agent, wherein after the composition is stored for a period of about 24 weeks at a temperature of about 40° C., a decrease between an aggregate chromatogram peak area for the stable liquid pharmaceutical composition comprising monoclonal human IgG2 antibodies and the chelating agent; and an aggregate chromatogram peak area for an otherwise identical composition lacking the chelating agent that is stored for a period of about 24 weeks at a temperature of about 40° C., is at least about 2%.

BRIEF DESCRIPTION OF THE DRAWINGS

[0021] FIG. **1** shows a line graph that shows the percent fragmentation in SDS-PAGE reduced gels with anti-M-CSF antibody 8.10.3F compositions stored for six weeks at 40° C.;

[0022] FIG. **2** shows a line graph that shows the percent major (isoelectric focusing) IEF band in IEF gels with anti-M-CSF antibody 8.10.3F compositions stored for six weeks at 40° C.;

[0023] FIG. **3** shows a line graph that shows the percent aggregation determined from SE-HPLC data with anti-M-CSF antibody 8.10.3F compositions stored for six weeks at 40° C.;

[0024] FIG. **4** shows a line graph that shows the percent aggregation determined from SE-HPLC data with anti-M-CSF antibody 8.10.3F compositions stored for 26 weeks at 40° C.;

[0025] FIG. **5** shows a line graph that shows the percent fragmentation (approximately 11 kD) determined from organic SE-HPLC data with anti-M-CSF antibody 8.10.3F compositions stored for up to 26 weeks at 40° C.;

[0026] FIG. **6** shows a line graph that shows the percent fragmentation from SDS-PAGE reduced gels with anti-M-CSF antibody 8.10.3F compositions stored for 26 weeks at 40° C.;

[0027] FIG. **7** shows a line graph that shows the percentage of remaining antibody monomer from SDS-PAGE non-reduced gels with anti-M-CSF antibody 8.10.3F compositions stored for 26 weeks at 40° C.;

[0028] FIG. **8** shows an organic SE-HPLC chromatogram (shown expanded in y-axis) of anti-M-CSF antibody 8.10.3F compositions 11 and 9 stored for 26 weeks at 40° C.;

[0029] FIG. **9** shows a photograph of a reduced SDS-PAGE gel of anti-M-CSF antibody 8.10.3F compositions stored for six weeks at 40° C.;

[0030] FIG. **10** shows an organic SE-HPLC chromatogram of anti-M-CSF antibody 8.10.3F in composition 1 (top) stored at 40° C. for 6 weeks compared to a control sample (bottom).

[0031] FIG. **11** shows an SE-HPLC chromatogram for anti-M-CSF antibody 8.10.3F stored in composition 11 for 26 weeks at 40° C.;

[0032] FIG. **12**, comprising FIGS. **12**A-**12**D, shows the nucleic acid and amino acid sequences for anti-M-CSF antibody 8.11.3F. FIG. **12**A shows the full-length nucleic acid sequence for the 8.11.3F heavy chain (SEQ ID NO: 1). FIG. **12**B shows the full-length amino acid sequence for the 8.11.3F heavy chain (SEQ ID NO: 2), and the amino acid sequence for the 8.11.3F heavy chain variable region is in upper case and designated between brackets "[ ]" (SEQ ID NO: 5). The amino acid sequence of each 8.11.3F heavy chain CDR is underlined and in lowercase. The heavy chain CDR amino acid sequences are as follows: CDR1: GFTFSSFSMT (SEQ ID NO: 7); CDR2: YISSRSSTISYADSVKG (SEQ ID NO: 8); and CDR3: DPLLAGATFFDY (SEQ ID NO: 9). FIG. **12**C shows the nucleic acid sequence for the full-length 8.11.3F light chain (SEQ ID NO: 3). FIG. **12**D shows the amino acid sequence of the full-length 8.11.3F light chain (SEQ ID NO: 4), and the 8.11.3F light chain variable region is in upper case and designated between brackets "[ ]" (SEQ ID NO: 6). The amino acid sequence of each light chain CDR is indicated as follows: CDR1: RASQSVSSSYLA (SEQ ID NO: 10); CDR2: GASSRAT (SEQ ID NO: 11); and CDR3: QQYGSSPLT (SEQ ID NO: 12);

[0033] FIG. **13** is a bar graph that shows the percent aggregation in various test compositions after storage at 40° C. for up to 7 weeks by size exclusion chromatography (SEC);

[0034] FIG. **14** is a bar graph that shows the percent total hydrolytic (fragmentation) impurities formation in various test compositions after storage at 40° C. for up to 7 weeks by reduced SDSPAGE (rSDSPAGE);

[0035] FIG. **15** is a line graph that shows the percent aggregation in various test compositions on storage under accelerated conditions at 40° C. for up to 24 weeks by SEC;

[0036] FIG. **16** is a line graph that shows the percent total hydrolytic (fragmentation) impurities formation in various test compositions on storage under accelerated conditions at 40° C. for up to 24 weeks by rSDSPAGE;

[0037] FIG. **17** is a line graph that shows the percent aggregation in various test compositions on storage under accelerated conditions at 40° C. for up to 24 weeks by SEC;

[0038] FIG. **18** is a line graph that shows the percent total hydrolytic (fragmentation) impurities formation in various test compositions on storage under accelerated conditions at 40° C. for up to 24 weeks by rSDSPAGE;

[0039] FIG. **19** is a bar graph that shows the percent aggregation in various test compositions as a function of EDTA level on storage under accelerated conditions at 40° C. for up to 24 weeks by SEC;

[0040] FIG. **20** is a bar graph that shows the percent total hydrolytic (fragmentation) impurities formation in various test compositions as a function of EDTA level on storage under accelerated conditions at 40° C. for up to 24 weeks by rSDSPAGE;

[0041] FIG. **21** is a line graph that shows the percent aggregation in various test compositions from on storage under accelerated conditions at 40° C. for up to 13 weeks by SEC;

[0042] FIG. **22** is a line graph that shows the percent total hydrolytic (fragmentation) impurities formation in various test compositions from on storage under accelerated conditions at 40° C. for up to 13 weeks by rSDSPAGE;

[0043] FIG. **23** comprising FIGS. **23**A-**23**D, shows the nucleotide and amino acid sequences for anti-CTLA4 antibody 11.2.1, now referred to as ticilimumab. FIG. **11**A shows the full length nucleotide sequence for the 11.2.1 heavy chain (SEQ ID NO: 21). FIG. **11**B shows the full length amino acid sequence for the 11.2.1 heavy chain (SEQ ID NO: 22), and the amino acid sequence for the 11.2.1 heavy chain variable region as indicated between brackets "[ ]" (SEQ ID NO: 25). The amino acid sequence of each 11.2.1 heavy chain CDR is underlined. The CDR sequences are as follows: CDR1: GFTFSSYGMH (SEQ ID NO: 27); CDR2: VIWYDGSNKYYADSV (SEQ ID NO: 28); and CDR3: DPRGATLYYYYYGMDV (SEQ ID NO: 29). FIG. **23**C shows the nucleotide sequence for the 11.2.1 light chain (SEQ ID NO:

23). FIG. **23**D shows the amino acid sequence of the full-length 11.2.1 light chain (SEQ ID NO: 24), and the light chain variable region as indicated between brackets "[ ]" (SEQ ID NO: 26). The amino acid sequence of each CDR is indicated as follows: CDR1: RASQSINSYLD (SEQ ID NO: 30); CDR2: AASSLQS (SEQ ID NO: 31); and CDR3: QQYYSTPFT (SEQ ID NO: 32);

[0044] FIG. **24** shows a graph that illustrates the percent aggregation in various test compositions differing in monoclonal antibody concentration after storage at 40° C. for up to 26 weeks by SEC;

[0045] FIG. **25** shows a graph that illustrates the percent aggregation in various test compositions differing in EDTA concentration after storage at 40° C. for up to 26 weeks, by SEC;

[0046] FIG. **26** shows a graph that illustrates the percent aggregation in various test compositions differing in polysorbate 80 concentration after storage at 40° C. for up to 26 weeks by SEC;

[0047] FIG. **27** shows a graph that illustrates the percent aggregation in various test compositions differing in buffer species after storage at 40° C. for up to 26 weeks by SEC;

[0048] FIG. **28** shows a graph that illustrates the percent aggregation in various test compositions differing in stabilizer/tonicifier species after storage at 40° C. for up to 26 weeks by SEC;

[0049] FIG. **29** shows a graph that illustrates the percent aggregation in various test compositions differing in surfactant species after storage at 40° C. for up to 26 weeks by SEC; and

[0050] FIG. **30** comprising FIGS. **30**A-**30**E, shows the nucleotide and amino acid sequences for anti-MAdCAM antibody 7.16.6. FIG. **30**A shows the nucleotide sequence for the 7.16.6 heavy chain (SEQ ID NO: 41). FIG. **30**B shows the amino acid sequence of the 7.16.6 heavy chain (SEQ ID NO: 42). FIG. **30**C shows the nucleotide sequence for the 7.16.6 light chain (SEQ ID NO: 43). FIG. **30**D shows the amino acid sequence of the full-length 7.16.6 light chain (SEQ ID NO: 44). FIG. **30**E shows the amino acid sequence of a MAdCAM-IgG_1 Fc Fusion Protein (SEQ ID NO: 45).

DETAILED DESCRIPTION OF THE INVENTION

[0051] The methods and techniques of the present invention are generally performed according to conventional methods well known in the art and as described in various general and more specific references that are cited and discussed throughout the present specification unless otherwise indicated. See, e.g., Sambrook et al., *Molecular Cloning: A Laboratory Manual,* 2d ed., Cold Spring Harbor Laboratory Press, Cold Spring Harbor, N.Y. (1989) and Ausubel et al., *Current Protocols in Molecular Biology*, Greene Publishing Associates (1992), and Harlow and Lane *Antibodies: A Laboratory Manual*, Cold Spring Harbor Laboratory Press, Cold Spring Harbor, N.Y. (1990). Enzymatic reactions and purification techniques are performed according to manufacturer's specifications, as commonly accomplished in the art or as described herein. The nomenclatures used in connection with, and the laboratory procedures and techniques of, analytical chemistry, synthetic organic chemistry, and medicinal and pharmaceutical chemistry described herein are those well known and commonly used in the art. Standard techniques are used for chemical syntheses, chemical analyses, pharmaceutical preparation, composition, and delivery, and treatment of subjects.

DEFINITIONS

[0052] In order to aid the reader in understanding the following detailed description, the following definitions are provided:

[0053] As used herein, the term "antibody" refers to an intact antibody or an antigen-binding portion that competes with the intact antibody for specific binding. See generally, *Fundamental Immunology*, Ch. 7 (Paul, W., ed., 2nd ed. Raven Press, N.Y. (1989). Antigen-binding portions may be produced by recombinant DNA techniques or by enzymatic or chemical cleavage of intact antibodies. In some embodiments, antigen-binding portions include Fab, Fab', $F(ab')_2$, Fd, Fv, dAb, and complementarity determining region (CDR) fragments, single-chain antibodies (scFv), chimeric antibodies, diabodies and polypeptides that contain at least a portion of an antibody that is sufficient to confer specific antigen binding to the polypeptide. From N-terminus to C-terminus, both the mature light and heavy chain variable domains comprise the regions FR1, CDR1, FR2, CDR2, FR3, CDR3 and FR4. The assignment of amino acids to each domain is in accordance with the definitions of Kabat, *Sequences of Proteins of Immunological Interest* (National Institutes of Health, Bethesda, Md. (1987 and 1991)), Chothia & Lesk, *J. Mol. Biol.* 196:901-917 (1987), or Chothia et al., *Nature* 342:878-883 (1989).

[0054] In some embodiments, the antibody is a single-chain antibody (scFv) in which a V_L and V_H domains are paired to form a monovalent molecules via a synthetic linker that enables them to be made as a single protein chain. (Bird et al., *Science* 242:423-426 (1988) and Huston et al., *Proc. Natl. Acad. Sci. USA* 85:5879-5883 (1988).) In some embodiments, the antibodies are diabodies, i.e., are bivalent antibodies in which V_H and V_L domains are expressed on a single polypeptide chain, but using a linker that is too short to allow for pairing between the two domains on the same chain, thereby forcing the domains to pair with complementary domains of another chain and creating two antigen binding sites. (See e.g., Holliger P. et al, *Proc. Natl. Acad. Sci. USA* 90:6444-6448 (1993), and Poljak R. J. et al., *Structure* 2:1121-1123 (1994)). In some embodiments, one or more CDRs from an antibody of the invention may be incorporated into a molecule either covalently or noncovalently to make it an immunoadhesin that specifically binds to the desired human antigen. In such embodiments, the CDR(s) may be incorporated as part of a larger polypeptide chain, may be covalently linked to another polypeptide chain, or may be incorporated noncovalently. While DNA sequences identifying a signal polypeptide may be identified in the sequence identifiers (SEQ ID NOS) herein, the antibodies typically do not comprise a signal polypeptide because the signal polypeptide is generally eliminated during post-translational modifications. In various embodiments of the invention, one or both of the heavy and light chains of the human IgG2 antibodies includes a signal sequence (or a portion of the signal sequence). In other embodiments of the invention, neither the heavy nor light chain of the human IgG2 antibodies includes a signal sequence.

[0055] As used herein, an antibody that is referred to by number is the same as a monoclonal antibody that is obtained from the hybridoma of the same number. For example, mono-

clonal antibody 8.10.3F is the same antibody as one obtained from hybridoma 8.10.3F. Thus, monoclonal antibody 8.10.3F has the same heavy and light chain amino acid sequences as one obtained from hybridoma 8.10.3F. Thus, reference to antibody 8.10.3F includes an antibody, which has the heavy and light chain amino acid sequences shown in SEQ ID NOS. 2 and 4, respectively. It also includes an antibody lacking a terminal lysine on the heavy chain, as this is normally lost in a proportion of antibodies during manufacture.

[0056] As used herein, an Fd fragment means an antibody fragment that consists of the V_H and C_H1 domains; an Fv fragment consists of the V_L and V_H domains of a single arm of an antibody; and a dAb fragment (Ward et al., *Nature* 341: 544-546 (1989)) consists of a V_H domain.

[0057] As used herein, the term "polypeptide" encompasses native or artificial proteins, protein fragments and polypeptide analogs of a protein sequence. A polypeptide may be monomeric or polymeric.

[0058] The terms "or an antigen-binding portion thereof" when used with the term "antibody" refers to a polypeptide that has an amino-terminal and/or carboxy-terminal deletion, but where the remaining amino acid sequence is identical to the corresponding positions in the naturally-occurring sequence. In some embodiments, the antigen-binding portion thereof may be at least 14, at least 20, at least 50, or at least 70, 80, 90, 100, 150 or at least 200 amino acids long.

[0059] As used herein, the terms "is capable of specifically binding" refers to when an antibody binds to an antigen with a dissociation constant that is ≦1 μM, preferably ≦1 nM and most preferably ≦10 pM. In certain embodiments, the K_D is 1 pM to 500 pM. In other embodiments, the K_D is between 500 pM to 1 μM. In other embodiments, the K_D is between 1 μM to 100 nM. In other embodiments, the K_D is between 100 mM to 10 nM.

[0060] As used herein, the term "monoclonal antibody" refers to an antibody obtained from a population of substantially homogeneous antibodies, i.e., the individual antibodies comprising the population are identical except for possible naturally occurring mutations that may be present in minor amounts or lacking a C-terminal lysine. Monoclonal antibodies are highly specific, being directed against a single antigenic site. Furthermore, in contrast to conventional (polyclonal) antibody preparations, which typically include different antibodies, directed against different determinants (epitopes), each monoclonal antibody is directed against a single determinant on the antigen. The modifier "monoclonal" indicates the character of the antibody as being obtained from a substantially homogeneous population of antibodies, and is not to be construed as requiring production of the antibody by any particular method. For example, the monoclonal antibodies to be used in accordance with the present invention may be made by the hybridoma method first described by Kohler, et al., *Nature* 256:495 (1975), or may be made by recombinant DNA methods (see, e.g., U.S. Pat. No. 4,816,567). The "monoclonal antibodies" may also be isolated from phage antibody libraries using the techniques described in Clackson, et al., *Nature* 352:624-628 (1991) and Marks, et al., *J. Mol. Biol.* 222:581-597 (1991), for example.

[0061] The term "isolated protein", "isolated polypeptide" or "isolated antibodies" is a protein, polypeptide or antibody that by virtue of its origin or source of derivation has one to four of the following: (1) is not associated with naturally associated components that accompany it in its native state, (2) is free of other proteins from the same species, (3) is expressed by a cell from a different species, or (4) does not occur in nature. Thus, a polypeptide that is chemically synthesized or synthesized in a cellular system different from the cell from which it naturally originates will be "isolated" from its naturally associated components. An isolated protein/antibody may also be rendered substantially free of naturally associated cellular components by isolation, using protein purification techniques well known in the art.

[0062] Examples of isolated/purified antibodies includes any human IgG2 antibody, such as an anti-CTLA-4 antibody, which has been affinity purified using an antigen, such as CTLA-4, and/or an anti-CTLA-4 antibody that has been synthesized by a hybridoma or other cell line in vitro, and/or a human anti-CTLA-4 antibody derived from a transgenic mouse. Thus, in preferred embodiments, the anti-CTLA-4 antibodies have a purity of at least about 95% (w/w—weight anti-CTLA-4 antibodies/weight of components other than pharmaceutically acceptable excipients), and in further embodiments, the anti-CTLA-4 antibodies have a purity from about 95% w/w to about 99.5% w/w.

[0063] An antibody is "substantially pure," "substantially homogeneous," or "substantially purified" when at least about 60 to 75% of a sample exhibits a single species of antibody. The antibody may be monomeric or multimeric. A substantially pure antibody can typically comprise about 50%, 60%, 70%, 80% or 90% w/w of an antibody sample, more usually about 95%, and preferably will be over 99% pure. Antibody purity or homogeneity may be indicated by a number of means well known in the art, such as polyacrylamide gel electrophoresis of an antibody sample, followed by visualizing a single polypeptide band upon staining the gel with a stain well known in the art. For certain purposes, higher resolution may be achieved by using HPLC or other means well known in the art for purification.

[0064] As used herein, the term "human antibody" is intended to include antibodies having variable and constant regions derived from human germline immunoglobulin sequences. The human antibodies of the invention may include amino acid residues not encoded by human germine immunoglobulin sequences (e.g., mutations introduced by random or site-specific mutagenesis in vitro or by somatic mutation in vivo), for example in the CDRs and in particular CDR3. However, the term "human antibody", as used herein, is not intended to include antibodies in which CDR sequences derived from the germline of another mammalian species, such as a mouse, have been grafted onto human framework sequences.

[0065] As used herein, the term "recombinant human antibody" is intended to include all human antibodies that are prepared, expressed, created or isolated by recombinant means, such as antibodies expressed using a recombinant expression vector transfected into a host cell, antibodies isolated from a recombinant, combinatorial human antibody library, antibodies isolated from an animal (e.g., a mouse) that is transgenic for human immunoglobulin genes (see e.g., Taylor, L. D., et al. (1992) *Nucl. Acids Res.* 20:6287-6295) or antibodies prepared, expressed, created or isolated by any other means that involves splicing of human immunoglobulin gene sequences to other DNA sequences. Such recombinant human antibodies have variable and constant regions derived from human germline immunoglobulin sequences. In certain embodiments, however, such recombinant human antibodies are subjected to in vitro mutagenesis (or, when an animal transgenic for human Ig sequences is used, in vivo somatic

mutagenesis) and thus the amino acid sequences of the V_H and V_L regions of the recombinant antibodies are sequences that, while derived from and related to human germline V_H and V_L sequences, may not naturally exist within the human antibody germline repertoire in vivo.

[0066] The term "epitope" includes any protein determinant capable of specific binding to an immunoglobulin or T-cell receptor or otherwise interacting with a molecule. Epitopic determinants generally consist of chemically active surface groupings of molecules such as amino acids or sugar side chains and generally have specific three-dimensional structural characteristics, as well as specific charge characteristics. An epitope may be "linear" or "conformational." In a linear epitope, all of the points of interaction between the protein and the interacting molecule (such as an antibody) occur linearly along the primary amino acid sequence of the protein. In a conformational epitope, the points of interaction occur across amino acid residues on the protein that are separated from one another.

[0067] As used herein, the term "polynucleotide" or "nucleic acid", used interchangeably herein, means a polymeric form of nucleotides of at least 10 bases in length, either ribonucleotides or deoxynucleotides or a modified form of either type of nucleotide. The term includes single and double stranded forms. A "polynucleotide" or a "nucleic acid" sequence encompasses its complement unless otherwise specified. Thus, a reference to a nucleic acid having a particular sequence should be understood to encompass its complementary strand, with its complementary sequence.

[0068] As used herein, the term "isolated polynucleotide" or "isolated nucleic acid" means a polynucleotide of genomic, cDNA, or synthetic origin or some combination thereof, which by virtue of its origin or source of derivation, the isolated polynucleotide has one to three of the following: (1) is not associated with all or a portion of a polynucleotide with which the "isolated polynucleotide" is found in nature, (2) is operably linked to a polynucleotide to which it is not linked in nature, or (3) does not occur in nature as part of a larger sequence.

[0069] The term "oligonucleotide" as used herein includes naturally occurring, and modified nucleotides linked together by naturally occurring and non-naturally occurring oligonucleotide linkages. Oligonucleotides are a polynucleotide subset generally comprising a length of 200 bases or fewer. Preferably oligonucleotides are 10 to 60 bases in length and most preferably 12, 13, 14, 15, 16, 17, 18, 19, or 20 to 40 bases in length. Oligonucleotides are usually single stranded, e.g. for primers and probes; although oligonucleotides may be double stranded, e.g. for use in the construction of a gene mutant. Oligonucleotides of the invention can be either sense or antisense oligonucleotides.

[0070] As used herein, the term "naturally occurring nucleotides" includes deoxyribonucleotides and ribonucleotides. The term "modified nucleotides" as used herein includes nucleotides with modified or substituted sugar groups and the like. The term "oligonucleotide linkages" referred to herein includes oligonucleotides linkages such as phosphorothioate, phosphorodithioate, phosphoroselenoate, phosphorodiselenoate, phosphoroanilothioate, phoshoraniladate, phosphoroamidate, and the like. See e.g., LaPlanche et al., *Nucl. Acids Res.* 14:9081 (1986); Stec et al., *J. Am. Chem. Soc.* 106:6077 (1984); Stein et al., *Nucl. Acids Res.* 16:3209 (1988); Zon et al., *Anti-Cancer Drug Design* 6:539 (1991); Zon et al., *Oligonucleotides and Analogues: A Practical Approach*, pp. 87-108 (F. Eckstein, Ed., Oxford University Press, Oxford England (1991)); U.S. Pat. No. 5,151,510; Uhlmann and Peyman, *Chemical Reviews* 90:543 (1990). An oligonucleotide can include a label for detection, if desired.

[0071] As used herein, the terms "selectively hybridize" mean to detectably and specifically bind. Polynucleotides, oligonucleotides and fragments thereof in accordance with the invention selectively hybridize to nucleic acid strands under hybridization and wash conditions that minimize appreciable amounts of detectable binding to nonspecific nucleic acids. "High stringency" or "highly stringent" conditions can be used to achieve selective hybridization conditions as known in the art and discussed herein. One example of "high stringency" or "highly stringent" conditions is the incubation of a polynucleotide with another polynucleotide, wherein one polynucleotide may be affixed to a solid surface such as a membrane, in a hybridization buffer of 6×SSPE or SSC, 50% formamide, 5×Denhardt's reagent, 0.5% SDS, 100 μg/ml denatured, fragmented salmon sperm DNA at a hybridization temperature of 42° C. for 12-16 hours, followed by twice washing at 55° C. using a wash buffer of 1×SSC, 0.5% SDS. See also Sambrook et al., supra, pp. 9.50-9.55.

[0072] As applied to polynucleotides, the terms "substantial identity", "percent identity" or "% identical" mean the percent of residues when a first contiguous sequence is compared and aligned for maximum correspondence to a second contiguous sequence. The length of sequence identity comparison may be over a stretch of at least about nine nucleotides, usually at least about 18 nucleotides, more usually at least about 24 nucleotides, typically at least about 28 nucleotides, more typically at least about 32 nucleotides, and preferably at least about 36, 48 or more nucleotides. The terms "substantial identity", "percent identity" or "% identical" mean that when a polynucleotide molecule is optimally aligned with appropriate nucleotide insertions or deletions with another polynucleotide molecule (or its complementary strand), there is polynucleotide sequence identity of at least about 85%, preferably at least about 90%, and more preferably at least about 95%, 96%, 97%, 98% or 99% of the nucleotide bases, as measured by any well-known algorithm of sequence identity, such as FASTA, BLAST or Gap. There are a number of different algorithms known in the art that can be used to measure polynucleotide sequence identity. For instance, polynucleotide sequences can be compared using FASTA, Gap or Bestfit, which are programs in Wisconsin Package Version 10.0, Genetics Computer Group (GCG), Madison, Wis. FASTA, which includes, e.g., the programs FASTA2 and FASTA3, provides alignments and percent sequence identity of the regions of the best overlap between the query and search sequences (Pearson, *Methods Enzymol.* 183:63-98 (1990); Pearson, *Methods Mol. Biol.* 132:185-219 (2000); Pearson, *Methods Enzymol.* 266:227-258 (1996); Pearson, *J. Mol. Biol.* 276:71-84 (1998)). Unless otherwise specified, default parameters for a particular program or algorithm are used. For instance, percent sequence identity between nucleic acid sequences can be determined using FASTA with its default parameters (a word size of 6 and the NOPAM factor for the scoring matrix) or using Gap with its default parameters as provided in GCG Version 6.1, herein incorporated by reference.

[0073] As applied to polypeptides, the terms "substantial identity", "percent identity" or "% identical" mean that two peptide sequences, when optimally aligned, such as by the programs GAP or BESTFIT using default gap weights, as

supplied with the programs, share at least 70%, 75% or 80% sequence identity, preferably at least 90% or 95% sequence identity, and more preferably at least 97%, 98% or 99% sequence identity. In certain embodiments, residue positions that are not identical differ by conservative amino acid substitutions. A "conservative amino acid substitution" is one in which an amino acid residue is substituted by another amino acid residue having a side chain R group with similar chemical properties (e.g., charge or hydrophobicity). In general, a conservative amino acid substitution will not substantially change the functional properties of a protein. In cases where two or more amino acid sequences differ from each other by conservative substitutions, the percent sequence identity may be adjusted upwards to correct for the conservative nature of the substitution. Means for making this adjustment are well-known to those of skill in the art. See, e.g., Pearson, *Methods Mol. Biol.* 243:307-31 (1994). Examples of groups of amino acids that have side chains with similar chemical properties include 1) aliphatic side chains: glycine, alanine, valine, leucine, and isoleucine; 2) aliphatic-hydroxyl side chains: serine and threonine; 3) amide-containing side chains: asparagine and glutamine; 4) aromatic side chains: phenylalanine, tyrosine, and tryptophan; 5) basic side chains: lysine, arginine, and histidine; 6) acidic side chains: aspartic acid and glutamic acid; and 7) sulfur-containing side chains: cysteine and methionine. Conservative amino acids substitution groups are: valine-leucine-isoleucine, phenylalanine-tyrosine, lysine-arginine, alanine-valine, glutamate-aspartate, and asparagine-glutamine. Sequence identity for polypeptides, is typically measured using sequence analysis software. Protein analysis software matches sequences using measures of similarity assigned to various substitutions, deletions and other modifications, including conservative amino acid substitutions. For instance, GCG contains programs such as "Gap" and "Bestfit" which can be used with default parameters, as specified with the programs, to determine sequence homology or sequence identity between closely related polypeptides, such as homologous polypeptides from different species of organisms or between a wild type protein and a mutant thereof. See, e.g., GCG Version 6.1. Polypeptide sequences also can be compared using FASTA using default or recommended parameters, see GCG Version 6.1. (University of Wisconsin WI) FASTA (e.g., FASTA2 and FASTA3) provides alignments and percent sequence identity of the regions of the best overlap between the query and search sequences (Pearson, *Methods Enzymol.* 183:63-98 (1990); Pearson, *Methods Mol. Biol.* 132:185-219 (2000)). Another preferred algorithm when comparing a sequence of the invention to a database containing a large number of sequences from different organisms is the computer program BLAST, especially blastp or tblastn, using default parameters, as supplied with the programs. See, e.g., Altschul et al., *J. Mol. Biol.* 215:403-410 (1990); Altschul et al., *Nucleic Acids Res.* 25:3389-402 (1997). The length of polypeptide sequences compared for homology will generally be at least about 16 amino acid residues, usually at least about 20 residues, more usually at least about 24 residues, typically at least about 28 residues, and preferably more than about 35 residues. When searching a database containing sequences from a large number of different organisms, it is preferable to compare amino acid sequences.

[0074] "Operably linked" sequences include both expression control sequences that are contiguous with the gene of interest and expression control sequences that act in trans or at a distance to control the gene of interest. The term "expression control sequence" as used herein means polynucleotide sequences that are necessary to effect the expression and processing of coding sequences to which they are ligated. Expression control sequences include appropriate transcription initiation, termination, promoter and enhancer sequences; efficient RNA processing signals such as splicing and polyadenylation signals; sequences that stabilize cytoplasmic mRNA; sequences that enhance translation efficiency (i.e., Kozak consensus sequence); sequences that enhance protein stability; and when desired, sequences that enhance protein secretion. The nature of such control sequences differs depending upon the host organism; in prokaryotes, such control sequences generally include promoter, ribosomal binding site, and transcription termination sequence; in eukaryotes, generally, such control sequences include promoters and transcription termination sequence. The term "control sequences" is intended to include, at a minimum, all components whose presence is essential for expression and processing, and can also include additional components whose presence is advantageous, for example, leader sequences and fusion partner sequences.

[0075] As used herein, the term "vector" means a nucleic acid molecule capable of transporting another nucleic acid to which it has been linked. In some embodiments, the vector is a plasmid, i.e., a circular double stranded DNA loop into which additional DNA segments may be ligated. In some embodiments, the vector is a viral vector, wherein additional DNA segments may be ligated into the viral genome. In some embodiments, the vectors are capable of autonomous replication in a host cell into which they are introduced (e.g., bacterial vectors having a bacterial origin of replication and episomal mammalian vectors). In other embodiments, the vectors (e.g., non-episomal mammalian vectors) can be integrated into the genome of a host cell upon introduction into the host cell, and thereby are replicated along with the host genome. Moreover, certain vectors are capable of directing the expression of genes to which they are operatively linked. Such vectors are referred to herein as "recombinant expression vectors" (or simply, "expression vectors").

[0076] As used herein, the terms "recombinant host cell" (or simply "host cell") means a cell into which a recombinant expression vector has been introduced. It should be understood that "recombinant host cell" and "host cell" mean not only the particular subject cell but also the progeny of such a cell. Because certain modifications may occur in succeeding generations due to either mutation or environmental influences, such progeny may not, in fact, be identical to the parent cell, but are still included within the scope of the term "host cell" as used herein.

[0077] A "therapeutically effective amount" refers to an amount effective, at dosages and for periods of time necessary, to achieve the desired therapeutic result, which includes treatment or prophylactic prevention of any condition, including inflammatory diseases and neoplasia disorders. It is to be noted that dosage values may vary with the severity of the condition to be alleviated. It is to be further understood that for any particular subject, specific dosage regimens should be adjusted over time according to the individual need and the professional judgment of the person administering or supervising the administration of the compositions, and that dosage ranges set forth herein are exemplary only and are not intended to limit the scope or practice of the claimed composition. Likewise, a therapeutically effective amount of the

antibody or antibody portion may vary according to factors such as the disease state, age, sex, and weight of the individual, the ability of the antibody or antibody portion to elicit a desired response in the individual, and the desired route of administration of the antibody composition. A therapeutically effective amount is also one in which any toxic or detrimental effects of the antibody or antibody portion are outweighed by the therapeutically beneficial effects.

[0078] As used herein, the term "subject" for purposes of treatment includes any subject, and preferably is a subject who is in need of the treatment of an inflammatory or neoplasia disorder. For purposes of prevention, the subject is any subject, and preferably is a subject that is at risk for, or is predisposed to, developing an inflammatory or neoplasia disorder. The term "subject" is intended to include living organisms, e.g., prokaryotes and eukaryotes. Examples of subjects include mammals, e.g., humans, dogs, cows, horses, pigs, sheep, goats, cats, mice, rabbits, rats, and transgenic non-human animals. In specific embodiments of the invention, the subject is a human.

[0079] As used herein, the terms "neoplasia" and "neoplasia disorders", used interchangeably herein, refer to new cell growth that results from a loss of responsiveness to normal growth controls, e.g. to "neoplastic" cell growth. Neoplasia is also used interchangeably herein with the term "cancer" and for purposes of the present invention; cancer is one subtype of neoplasia. As used herein, the term "neoplasia disorder" also encompasses other cellular abnormalities, such as hyperplasia, metaplasia and dysplasia. The terms neoplasia, metaplasia, dysplasia and hyperplasia can be used interchangeably herein and refer generally to cells experiencing abnormal cell growth.

[0080] As used herein, the term "treatment" refers to both therapeutic treatment and prophylactic or preventative measures, wherein the object is to prevent or slow down (lessen) the targeted pathologic condition or condition. Those in need of treatment include those already with the condition as well as those prone to have the condition or those in whom the condition is to be prevented.

[0081] When introducing elements of the present invention or the preferred embodiment(s) thereof, the articles "a", "an", "the" and "said" are intended to mean that there are one or more of the elements. Throughout this specification and claims, the terms "comprising", "comprise", "comprises", "including" and "having" are intended to be inclusive and mean that there may be additional elements other than the listed elements.

Human IgG2 Antibodies:

[0082] In accordance with the present invention, it has been discovered that the stability of monoclonal human IgG2 antibodies can be improved by mixing the antibodies with a chelating agent, such as ethylenediaminetetraacetic acid ("EDTA").

[0083] While not wishing to be bound by theory, it is believed that the presence of a chelating agent in the compositions of the present invention help to improve stability of the antibody polypeptide by reducing the incidence of one or more of the following: antibody aggregation, fragmentation, oxidation, freeze/thaw instability, discoloration, and/or deamidation. The present invention comprises human IgG2 antibody compositions having improved chemical and/or physical stability as compared to previously disclosed antibody compositions.

[0084] Therefore, in certain aspects, the present invention provides a composition comprising a chelating agent, such as EDTA and a monoclonal"human IgG2 antibody" or an antigen-binding portion thereof. In still other aspects, the aforementioned human IgG2 antibody compositions comprising a chelating agent can include additional pharmaceutically acceptable excipients, including, but not limited to, one or more excipients that are chosen from buffers, tonicity agents, surfactants, antioxidants, and mixtures thereof.

[0085] As it will be appreciated; it is generally not desirable to kill antigen expressing cells. Rather, one generally desires to simply inhibit antigen binding. One of the major mechanisms through which antibodies kill cells is through fixation of complement and participation in CDC. The constant region of an antibody plays an important role in connection with an antibody's ability to fix complement and participate in CDC. Thus, generally one selects the isotype of an antibody to either provide the ability of complement fixation, or not. In the case of the present invention, generally, as mentioned above, it is generally not preferred to utilize an antibody that kills the cells. There are a number of isotypes of antibodies that are capable of complement fixation and CDC, including, without limitation, the following: murine IgM, murine IgG2a, murine IgG2b, murine IgG3, human IgM, human IgG1, and human IgG3. In contrast, a preferred isotype which is not capable of complement fixation and CDC include, without limitation, human IgG2. In addition to heavy chain sequence differences, the IgG antibodies differ within their subclass based on the number of disulfide bonds and length of the hinge region. For example, the IgG2 subclass has several differences distinct from the other subclasses. The IgG2 and IgG4 subclasses are known to have 4 disulfide bonds within their hinge region, while IgG1 has 2 and IgG3 has 11 disulfide bonds. Other differences for IgG2 antibodies include their reduced ability to cross the placenta and the inability of IgG2 antibodies to bind to lymphocyte Fc receptors. Thus, in certain embodiments, the human antibody is subclass IgG2.

[0086] As it will also be appreciated, it is generally desirable to utilize fully human antibodies in contrast to murine, chimeric or humanized antibodies. Antibodies which are not fully human have been associated with increased immunogenicity in human patients, which can lead to rapid clearance, reduced efficacy and an increased risk of infusion reactions, which can range from relatively benign fevers and rashes to cardiopulmonary and anaphylactic-like adverse events. See Lonberg, N., *Nature Biotechnology* 23, 1117-1125 (2005).

[0087] Several examples of human IgG2 antibodies that are suitable for use with the present invention, include, but are not limited to, the following antibodies.

Human IgG2 Anti-M-CSF Antibodies:

[0088] In certain embodiments, the human IgG2 antibodies that are suitable for use with the present invention include anti-M-CSF antibodies. Suitable anti-M-CSF antibodies and methods to prepare them that are described in U.S. Published Application No. 20050059113 to Bedian, et al. In other embodiments, the anti-M-CSF antibodies which are suitable for use with the present invention include any one or more of those anti-M-CSF monoclonal antibodies having the heavy and light chain amino acid sequences of the antibodies designated 252, 88, 100, 3.8.3, 2.7.3, 1.120.1, 9.14.41, 9.7.21F, 9.14.4, 8.10.3, 9.7.2, 9.7.2C-Ser, 9.14.4C-Ser, 8.10.3C-Ser, 8.10.3-CG2, 9.7.2-CG2, 9.7.2-CG4, 9.14.4-CG2, 9.14.4-CG4, 9.14.4-Ser, 9,7,2-Ser, 8.10.3-Ser, 8.10.3-CG4, 8.10.3FG1 or 9.14.4G1 in U.S. Published Application No. 20050059113 to Bedian, et al. In still other embodiments, the anti-M-CSF antibodies which are suitable for use with the present invention include those anti-M-CSF monoclonal antibodies having the heavy and light chain amino acid sequences

of the antibody designated 8.10.3F in U.S. Published Application No. 20050059113 to Bedian, et al.

[0089] In addition, such anti-M-CSF antibodies may be chosen based on differences in the amino acid sequences in the constant region of their heavy chains. For example, the anti-M-CSF antibodies may be chosen from the IgG2 class, which have "gamma" type heavy chains. The class and subclass of anti-M-CSF antibodies may be determined by any method known in the art. In general, the class and subclass of an antibody may be determined using antibodies that are specific for a particular class and subclass of antibody. Such antibodies are commercially available. The class and subclass can be determined by ELISA, or Western Blot as well as other techniques. Alternatively, the class and subclass may be determined by sequencing all or a portion of the constant domains of the heavy and/or light chains of the antibodies, comparing their amino acid sequences to the known amino acid sequences of various class and subclasses of immunoglobulins, and determining the class and subclass of the antibodies.

[0090] In other embodiments, suitable anti-M-CSF antibodies may be chosen based on differences in the amino acid sequences in their heavy chains. For example, the anti-M-CSF antibodies of the present invention may have human gamma type heavy chains that utilize any of the following human V_H germline genes: V_H1, V_H2, V_H3, V_H4, or V_H5. In certain embodiments, the anti-M-CSF antibodies utilize the human V_H3 germline gene. In further embodiments, the anti-M-CSF antibodies utilize the human V_H 3-48 germline gene. In still further embodiments, the anti-M-CSF antibodies utilize the D1-26 human DH gene. In still further embodiments, the anti-M-CSF antibodies utilize the J_H4 human J_H gene. For purposes of the present invention, the phrase "heavy chain variable region" is often abbreviated with the term (V_H).

[0091] In further embodiments, the anti-M-CSF antibodies may be chosen based on differences in the amino acid sequences of their light chains. For example, suitable anti-M-CSF antibodies may have lambda light chains or kappa light chains. However, in certain embodiments, the anti-M-CSF antibodies of the present invention have kappa light chains. In some embodiments, where the anti-M-CSF antibody comprises a kappa light chain, the polynucleotide encoding the variable domain of the light chain comprises a human V_KL5, O12, L2, B3, L15, or A27 gene and a human Jκ1, Jκ2, Jκ3, Jκ4, or Jκ5 gene. In some embodiments where the antibody comprises a kappa light chain, the light chain variable domain (V_L) is encoded in part by a human V_KA27 gene and a human J_K4 gene. In particular embodiments of the invention, the light chain variable domain is encoded by human V_KA27/J_K3 genes.

[0092] Table 1 lists the heavy chain and light chain human germline gene derivation and sequences for the anti-M-CSF monoclonal antibody 8.10.3F

TABLE 1

Heavy and Light Chain Human Gene Utilization and Sequences

	Heavy Chain				Light Chain		
Antibody	SEQ ID NO:	V_H	D_H	J_H	SEQ ID NO:	$V_κ$	$J_κ$
8.10.3F	1 (nucleic acid) 2 (amino acid)	3-48	1-26	4b	3 (nucleic acid) 4 (amino acid)	A27	4

[0093] Some anti-M-CSF antibodies in accordance with the present invention were generated with a bias towards the utilization of the human V_H 3-48 heavy chain variable region. In XenoMouse™ mice, there are more than 30 distinct functional heavy chain variable genes with which to generate antibodies. Bias, therefore, is indicative of a preferred binding motif of the antibody-antigen interaction with respect to the combined properties of binding to the antigen and functional activity.

[0094] In some embodiments, the nucleic acid molecule encodes an amino acid sequence comprising 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17 or 18 mutations compared to the germline amino acid sequence of the human V, D or J genes. In some embodiments, said mutations are in the heavy chain variable region. In some embodiments, said mutations are in the CDR regions.

[0095] In some embodiments, the nucleic acid molecule encodes one or more amino acid mutations compared to the germline sequence that are identical to amino acid mutations found in the V_H of monoclonal antibody 8.10.3F. In some embodiments, the nucleic acid encodes at least three amino acid mutations compared to the germline sequences that are identical to at least three amino acid mutations found in one of the above-listed monoclonal antibodies.

[0096] In some embodiments, the nucleic acid molecule encodes a V_L amino acid sequence comprising one or more variants compared to germline sequence that are identical to the variations found in the V_L of one of the antibodies 8.10.3F.

[0097] In some embodiments, the nucleic acid molecule encodes at least three amino acid mutations compared to the germline sequence found in the V_L of the antibody 8.10.3.

[0098] In some embodiments, the antibody is a single-chain antibody (scFv) in which a V_L and V_H domains are paired to form a monovalent molecules via a synthetic linker that enables them to be made as a single protein chain. Bird et al., *Science* 242:423-426 (1988) and Huston et al., *Proc. Natl. Acad. Sci. USA* 85:5879-5883 (1988). In some embodiments, the antibodies are diabodies, i.e., are bivalent antibodies in which V_H and V_L domains are expressed on a single polypeptide chain, but using a linker that is too short to allow for pairing between the two domains on the same chain, thereby forcing the domains to pair with complementary domains of another chain and creating two antigen binding sites. See e.g., Holliger P. et al., *Proc. Natl. Acad. Scl. USA* 90:6444-6448 (1993 and Poljak R. J. et al., *Structure* 2:1121-1123 (1994). In some embodiments, one or more CDRs from an antibody of the invention may be incorporated into a molecule either covalently or noncovalently to make it an immunoadhesin that specifically binds to M-CSF. In such embodiments, the CDR(s) may be incorporated as part of a larger polypeptide chain, may be covalently linked to another polypeptide chain, or may be incorporated noncovalently.

[0099] In another embodiment, the anti-M-CSF antibody has selectivity (or specificity) for M-CSF that is at least 100 times greater than its selectivity for any other polypeptide. In some embodiments, the anti-M-CSF antibody does not exhibit any appreciable specific binding to any other protein other than M-CSF. One can determine the selectivity of the anti-M-CSF antibody for M-CSF using methods well known in the art following the teachings of the specification. For instance, one can determine the selectivity using Western blot, FACS, ELISA, or RIA. Thus, in some embodiments, the monoclonal anti-M-CSF antibody is capable of specifically binding to the antigen, M-CSF.

[0100] In some embodiments, the C-terminal lysine of the heavy chain of the anti-M-CSF antibody of the invention is

not present. In various embodiments of the invention, the heavy and light chains of the anti-M-CSF antibodies may optionally include a signal sequence.

[0101] Table 1 lists the sequence identifiers (SEQ ID NOS) of the nucleic acids that comprise the heavy and light chains and the corresponding predicted amino acid sequences for the anti-M-CSF monoclonal antibody 8.10.3F. While DNA sequences encoding a signal polypeptide are shown in the sequence identifiers, the antibody typically does not comprise a signal polypeptide because the signal polypeptide is generally eliminated during post-translational modifications. In various embodiments of the invention, one or both of the heavy and light chains of the anti-M-CSF antibodies includes a signal sequence (or a portion of the signal sequence). In other embodiments of the invention, neither the heavy nor light chain of the anti-M-CSF antibodies includes a signal sequence.

[0102] In some embodiments, the nucleic acid molecule encodes a light chain amino acid sequence that is at least 70%, 75%, 80%, 85%, 90%, 95%, 97%, 98%, 99% or 100% identical to a light chain amino acid sequence of antibody 8.10.3F of SEQ ID NO: 4, or to a V_L amino acid sequence of SEQ ID NO 6. Nucleic acid molecules of the invention include nucleic acids that hybridize under highly stringent conditions, such as those described above, to a nucleic acid sequence encoding the light chain amino acid sequence of SEQ ID NO: 4, or that has the polynucleotide sequence of SEQ ID NO: 3.

[0103] In some embodiments, the nucleic acid molecule comprises a polynucleotide sequence that encodes the light chain amino acid sequence of monoclonal antibody 8.10.3F, or a portion thereof. In some embodiments, the nucleic acid molecule comprises a polynucleotide sequence that encodes the light chain polynucleotide sequence of monoclonal antibody 8.10.3F of SEQ ID NO: 3, or a portion thereof. In some embodiments, the nucleic acid molecule comprises a polynucleotide sequence that encodes the V_L amino acid sequence of monoclonal antibody 8.10.3F of SEQ ID NO: 6, or a portion thereof. In some embodiments, said portion comprises at least the CDR2 region. In some embodiments, the nucleic acid encodes the amino acid sequence of the light chain CDRs of said antibody. In some embodiments, said portion is a contiguous portion comprising CDR1-CDR3.

[0104] In some embodiments, the nucleic acid molecule encodes a heavy chain amino acid sequence that is at least 70%, 75%, 80%, 85%, 90%, 95%, 97%, 98%, 99% or 100% identical to a heavy chain amino acid sequence of antibody 8.10.3F of SEQ ID NO: 2, or to a V_H amino acid sequence of SEQ ID NO 5. Nucleic acid molecules of the invention include nucleic acids that hybridize under highly stringent conditions, such as those described above, to a nucleic acid sequence encoding the heavy chain amino acid sequence of SEQ ID NO: 2, or that has the polynucleotide sequence of SEQ ID NO: 1.

[0105] In some embodiments, the nucleic acid molecule comprises a polynucleotide sequence that encodes the heavy chain amino acid sequence of monoclonal antibody 8.10.3F, or a portion thereof. In some embodiments, the nucleic acid molecule comprises a polynucleotide sequence that encodes the heavy chain polynucleotide sequence of monoclonal antibody 8.10.3F of SEQ ID NO: 2, or a portion thereof. In some embodiments, the nucleic acid molecule comprises a polynucleotide sequence that encodes the V_H amino acid sequence of monoclonal antibody 8.10.3F of SEQ ID NO: 5, or a portion thereof. In some embodiments, said portion comprises at least the CDR2 region. In some embodiments, the nucleic acid encodes the amino acid sequence of the light chain CDRs of said antibody. In some embodiments, said portion is a contiguous portion comprising CDR1-CDR3.

[0106] In further embodiments, the nucleic acid molecule comprises a polynucleotide sequence that encodes at least a portion of the V_H amino acid sequence of 8.10.3F (SEQ ID NO: 5) or said sequence having conservative amino acid mutations and/or a total of three or fewer non-conservative amino acid substitutions. In various embodiments the sequence encodes one or more CDR regions, preferably a CDR3 region, all three CDR regions, a contiguous portion including CDR1-CDR3, or the entire V_H region.

[0107] In still further embodiments, the nucleic acid molecule comprises a polynucleotide sequence that encodes the heavy chain amino acid sequence of SEQ ID NO: 1 or a portion thereof. In still further embodiments, the nucleic acid molecule comprises a polynucleotide sequence that encodes the heavy chain variable domain amino acid sequence of SEQ ID NO: 5 or a portion thereof.

[0108] In another embodiment, the nucleic acid encodes a full-length light chain of an antibody selected from 8.10.3F, or a light chain comprising the amino acid sequence of SEQ ID NO: 4 and a constant region of a light chain, or a light chain comprising a mutation. Further, the nucleic acid may comprise the light chain polynucleotide sequence of SEQ ID NO: 3 and the polynucleotide sequence encoding a constant region of a light chain, or a nucleic acid molecule encoding a light chain comprise a mutation.

[0109] In some embodiments, the nucleic acid molecule comprises a polynucleotide sequence that encodes at least a portion of the V_H amino acid sequence of 8.10.3F (SEQ ID NO: 5) or said sequence having conservative amino acid mutations and/or a total of three or fewer non-conservative amino acid substitutions. In various embodiments the sequence encodes one or more CDR regions, preferably a CDR3 region, all three CDR regions, a contiguous portion including CDR1-CDR3, or the entire V_H region.

[0110] In another aspect of the invention, the anti-M-CSF antibodies demonstrate both species and molecule selectivity. In some embodiments, the anti-M-CSF antibody binds to human, cynomologus monkey and mouse M-CSF. Following the teachings of the specification, one may determine the species selectivity for the anti-M-CSF antibody using methods well known in the art. For instance, one may determine the species selectivity using Western blot, FACS, ELISA, RIA, a cell proliferation assay, or an M-CSF receptor-binding assay. In a preferred embodiment, one may determine the species selectivity using a cell proliferation assay or ELISA.

[0111] In another embodiment, the anti-M-CSF antibody has selectivity for M-CSF that is at least 100 times greater than its selectivity for GM-/G-CSF. In some embodiments, the anti-M-CSF antibody does not exhibit any appreciable specific binding to any other protein other than M-CSF. One can determine the selectivity of the anti-M-CSF antibody for M-CSF using methods well known in the art following the teachings of the specification. For instance one can determine the selectivity using Western blot, FACS, ELISA, or RIA.

Human IgG2 Anti-CTLA-4 Antibodies:

[0112] In other embodiments, the human IgG2 antibodies that are suitable for use with the present invention include anti-CTLA-4 antibodies. Suitable anti-CTLA-4 antibodies and methods to prepare them that are described in U.S. Pat.

No. 6,682,736 to Hanson, et al., filed on Dec. 23, 1999. In other embodiments, the anti-CTLA-4 antibodies which are suitable for use with the present invention include those anti-CTLA-4 monoclonal antibodies having the heavy and light chain amino acid sequences of the antibody designated 11.2.1 in U.S. Pat. No. 6,682,736. In other embodiments, the anti-CTLA-4 antibodies which are suitable for use with the present invention include those anti-CTLA-4 monoclonal antibodies having the heavy and light chain amino acid sequences of the antibodies ticilimumab and ipilimumab. In other embodiments, the anti-CTLA-4 antibodies which are suitable for use with the present invention include those anti-CTLA-4 monoclonal antibodies having the heavy and light chain amino acid sequences of the antibody ticilimumab.

[0113] As used herein, an antibody that is referred to by number has the same heavy and light chain amino acid sequences as a monoclonal antibody that is obtained from the hybridoma of the same number. For example, monoclonal antibody 11.2.1 has the same heavy and light chain amino acid sequences as one obtained from hybridoma 11.2.1. Thus, reference to antibody 11.2.1 includes the antibody, ticilimumabtm, which has the heavy and light chain amino acid sequences shown in SEQ ID NOS. 22 and 24, respectively, and the variable domain for the heavy chain shown in SEQ ID NO. 25 and the variable domain for the light chain shown in SEQ ID NO. 26. It also includes an antibody lacking a terminal lysine on the heavy chain, as this is normally lost in a proportion of antibodies during manufacture.

[0114] In addition, such anti-CTLA-4 antibodies may be chosen based on differences in the amino acid sequences in the constant region of their heavy chains. For example, the anti-CTLA-4 antibodies may be chosen from the IgG class, which have "gamma" type heavy chains. The class and subclass of anti-CTLA-4 antibodies may be determined by any method known in the art. In general, the class and subclass of an antibody may be determined using antibodies that are specific for a particular class and subclass of antibody. Such antibodies are commercially available. The class and subclass can be determined by ELISA, or Western Blot as well as other techniques. Alternatively, the class and subclass may be determined by sequencing all or a portion of the constant domains of the heavy and/or light chains of the antibodies, comparing their amino acid sequences to the known amino acid sequences of various class and subclasses of immunoglobulins, and determining the class and subclass of the antibodies.

[0115] In other embodiments, suitable anti-CTLA-4 antibodies may be chosen based on differences in the amino acid sequences in their heavy chains. For example, the anti-CTLA-4 antibodies of the present invention may have human gamma type heavy chains that utilize any of the following human V_H germline genes: V_H1, V_H2, V_H3, V_H4, or V_H5. In certain embodiments, the anti-CTLA-4 antibodies utilize the human V_H3 germline gene. In further embodiments, the anti-CTLA-4 antibodies utilize the human V_H3 germline gene and the human DP-50 or DP-46 heavy chain variable region, and in other embodiments, the anti-CTLA-4 antibodies utilize the human DP-50 heavy chain variable region. The DP-50 gene is also referred to as a V_H 3-33 family gene. The DP-46 gene is also referred to as a V_H 3-30.3 family gene. In still further embodiments, the anti-CTLA-4 antibodies utilize a human DH gene that is selected from D1-26, DIR4 and DIR3, and in other embodiments, the anti-CTLA-4 antibodies utilize a D1-26 human DH gene. In still further embodiments, the anti-CTLA-4 antibodies utilize a human J_H gene that is selected from J_H4 and J_H6, and in other embodiments, the anti-CTLA-4 antibodies utilize the J_H6 human J_H gene.

[0116] In further embodiments, the anti-CTLA-4 antibodies may be chosen based on differences in the amino acid sequences of their light chains. For example, suitable anti-CTLA-4 antibodies may have lambda light chains or kappa light chains. However, in certain embodiments, the anti-CTLA-4 antibodies of the present invention have kappa light chains. In some embodiments, where the anti-CTLA-4 antibody comprises a kappa light chain, the polynucleotide encoding the variable domain of the light chain comprises a human V_K L5, O12, L2, B3, L15, or A27 gene and a human Jκ1, Jκ2, Jκ3, Jκ4, or Jκ5 gene. In some embodiments where the antibody comprises a kappa light chain, the light chain variable domain (V_L) is encoded in part by a human V_KO12, or V_KA27 gene and a human J_K3 or J_K4 gene. In particular embodiments of the invention, the light chain variable domain is encoded by human V_K O12/Jκ3 genes.

[0117] Furthermore, the antibody can comprise a heavy chain amino acid sequence comprising human CDR amino acid sequences derived from the V_H 3-30 or 3-33 gene, or conservative substitutions or somatic mutations therein. It is understood that the V_H 3-33 gene encodes from FR1 through FR3 of the heavy chain variable region of an antibody molecule. Thus, the invention encompasses an antibody that shares at least 85%, more preferably, at least 90%, yet more preferably, at least 91%, even more preferably, at least 94%, yet more preferably, at least 95%, more preferably, at least 97%, even more preferably, at least 98%, yet more preferably, at least 99%, and most preferably, 100% identity, with the sequence from FR1 through FR3 of the antibody ticilimumab.

[0118] The antibody can further comprise CDR regions in its light chain derived from the A27 or the O12 gene or it may comprise the CDR regions of the antibody ticilimumab.

[0119] In other embodiments of the invention, the antibody inhibits binding between CTLA4 and B7-1, B7-2, or both. Preferably, the antibody can inhibit binding with B7-1 with an IC_{50} of about 100 nM or lower, more preferably, about 10 nM or lower, for example about 5 nM or lower, yet more preferably, about 2 nM or lower, or even more preferably, for example, about 1 nM or lower. Likewise, the antibody can inhibit binding with B7-2 with an IC_{50} of about 100 nM or lower, more preferably, 10 nM or lower, for example, even more preferably, about 5 nM or lower, yet more preferably, about 2 nM or lower, or even more preferably, about 1 nM or lower.

[0120] Further, in another embodiment, the anti-CTLA4 antibody has a binding affinity for CTLA4 of about 10^{-8}, or greater affinity, more preferably, about 10^{-9} or greater affinity, more preferably, about 10^{-10} or greater affinity, and even more preferably, about 10^{-11} or greater affinity.

[0121] The anti-CTLA4 antibody includes an antibody that competes for binding with an antibody having heavy and light chain amino acid sequences of the antibody ticilimumab. Further, the anti-CTLA4 antibody can compete for binding with antibody ipilimumab.

[0122] In another embodiment, the antibody preferably cross-competes with an antibody having a heavy and light chain sequence, a variable heavy and a variable light chain sequence, and/or the heavy and light CDR sequences of antibody ticilimumab. For example, the antibody can bind to the epitope to which an antibody that has heavy and light chain amino acid sequences, variable sequences and/or CDR

sequences, of the antibody ticilimumab binds. In another embodiment, the antibody cross-competes with an antibody having heavy and light chain sequences, or antigen-binding sequences, of MDX-D010.

[0123] In another embodiment, the invention is practiced using an anti-CTLA-4 antibody that comprises a heavy chain comprising the amino acid sequences of CDR-1, CDR-2, and CDR-3, and a light chain comprising the amino acid sequences of CDR-1, CDR-2, and CDR-3, of an antibody ticilimumab, or sequences having changes from the CDR sequences selected from the group consisting of conservative changes, wherein the conservative changes are selected from the group consisting of replacement of nonpolar residues by other nonpolar residues, replacement of polar charged residues other polar uncharged residues, replacement of polar charged residues by other polar charged residues, and substitution of structurally similar residues; non-conservative substitutions, wherein the non-conservative substitutions are selected from the group consisting of substitution of polar charged residue for polar uncharged residues and substitution of nonpolar residues for polar residues, additions and deletions.

[0124] In a further embodiment of the invention, the antibody contains fewer than 10, 7, 5, or 3 amino acid changes from the germline sequence in the framework or CDR regions. In another embodiment, the antibody contains fewer than 5 amino acid changes in the framework regions and fewer than 10 changes in the CDR regions. In one preferred embodiment, the antibody contains fewer than 3 amino acid changes in the framework regions and fewer than 7 changes in the CDR regions. In a preferred embodiment, the changes in the framework regions are conservative and those in the CDR regions are somatic mutations.

[0125] Even more preferably, the antibody shares 100% sequence identity or sequence similarity over the heavy chain and the light chain, or with the heavy chain or the light chain, separately, of an antibody ticilimumab.

[0126] In another embodiment, the antibody shares at least 80%, more preferably, at least 85%, even more preferably, at least 90%, yet more preferably, at least 94%, more preferably, at least, 95%, even more preferably, at least 99%, sequence identity or sequence similarity over the heavy and light chain full-length sequences, or over the heavy or the light chain, separately, with the sequences of germline V_K A27, germline V_{KO}12, and germline DP50 (which is an allele of the V_H 3-33 gene locus). Even more preferably, the antibody shares 100% sequence identity or sequence similarity over the heavy chain sequence of germline DP50 and/or with the light chain sequence of germline A27, or germline O12.

[0127] In one embodiment, the antibody shares at least 80%, more preferably, at least 85%, even more preferably, at least 90%, yet more preferably, at least 94%, preferably, at least 95%, more preferably, at least 99%, sequence (e.g., amino acid, nucleic acid, or both) identity or sequence similarity over the heavy and light chain variable region sequences, or over the heavy or the light chain variable region sequence, separately, with the sequences of antibody 3.1.1, 4.1.1, 4.8.1, 4.10.2, 4.13.1, 4.14.3, 6.1.1, ticilimumab, 11.6.1, 11.7.1, 12.3.1.1, 12.9.1.1, ipilimumab. Even more preferably, the antibody shares 100% sequence identity or sequence similarity over the heavy chain and the light chain variable region sequences, or with the heavy chain or the light chain sequence, separately, of an antibody selected from antibody 3.1.1, 4.1.1, 4.8.1, 4.10.2, 4.13.1, 4.14.3, 6.1.1, ticilimumab, 11.6.1, 11.7.1, 12.3.1.1, 12.9.1.1, ipilimumab.

[0128] In another embodiment, the antibody shares at least 80%, more preferably, at least 85%, even more preferably, at least 90%, yet more preferably, at least 94%, more preferably, at least 95%, even more preferably, at least 99%, sequence identity or sequence similarity over heavy chain variable region sequence with the heavy chain variable sequence of heavy germline DP50 (which is an allele of the V_H 3-33 gene locus) or with the light chain variable sequence of germline V_K A27, or germline V_K O12. Even more preferably, the antibody heavy chain region sequence shares 100% sequence identity or sequence similarity with the sequence of germline DP50 or with the light chain sequence of germline A27, or germline O12.

[0129] In one embodiment of the present invention, the antibody shares at least 80%, more preferably, at least 85%, even more preferably, at least 90%, yet more preferably, at least 95%, more preferably, at least 99%, sequence identity or sequence similarity with the heavy chain, the light chain, or both, sequences from FR1 through FR4 with the FR1 through FR4 region sequences of the antibody ticilimumab. Even more preferably, the antibody shares 100% sequence identity or sequence similarity over the heavy, light, or both, sequences from FR1 through FR4 with the antibody ticilimumab.

[0130] In another embodiment of the present invention, the antibody shares at least 80%, more preferably, at least 85%, even more preferably, at least 90%, yet more preferably, at least 95%, more preferably, at least 99%, and most preferably, about 100%, sequence identity or sequence similarity with the heavy chain sequences from FR1 through FR3 with the FR1 through FR3 region sequences of germline DP50.

[0131] In yet another embodiment of the present invention, the antibody shares at least 80%, more preferably, at least 85%, even more preferably, at least 90%, yet more preferably, at least 95%, more preferably, at least 99%, and most preferably, about 100%, sequence identity or sequence similarity with the light chain sequences from FR1 through FR4 with the FR1 through FR4 region sequences of germline V_K A27, or germline V_K O12.

[0132] In one embodiment of the present invention, the antibody shares at least 80%, more preferably, at least 85%, even more preferably, at least 90%, yet more preferably, at least 95%, more preferably, at least 99%, sequence identity or sequence similarity with the heavy chain, the light chain, or both, CDR-1, CDR-2 and CDR-3 sequences of the antibody ticilimumab. Even more preferably, the antibody shares 100% sequence identity or sequence similarity over the heavy, light, or both, CDR-1, CDR-2 and CDR-3 sequences with the antibody ticilimumab.

[0133] In another embodiment of the present invention, the antibody shares at least 80%, more preferably, at least 85%, even more preferably, at least 90%, yet more preferably, at least 95%, more preferably, at least 99%, and most preferably, about 100%, sequence identity or sequence similarity with the heavy chain CDR-1 and CDR-2 sequences with the CDR-1 and CDR-2 sequences of germline DP50.

[0134] In yet another embodiment of the present invention, the antibody shares at least 80%, more preferably, at least 85%, even more preferably, at least 90%, yet more preferably, at least 95%, more preferably, at least 99%, and most preferably, about 100%, sequence identity or sequence similarity

with the light chain CDR-1, CDR-2 and CDR-3 sequences with the CDR-1, CDR-2 and CDR-3 sequences of germline V_K A27, or germline V_K O12.

[0135] In one embodiment, the anti-CTLA-4 antibody is the antibody known as ticilimumab.

[0136] Table 2 lists the heavy chain and light chain human germline gene derivation for the anti-CTLA-4 monoclonal antibody 11.2.1 (i.e., ticilimumab).

TABLE 2

	Heavy Chain DNA				Light Chain DNA		
Clone	SEQ ID NO:	V_H	D_H	J_H	SEQ ID NO:	V_κ	J_κ
11.2.1	21 (cDNA) (full-length)	DP-50 (3-33)	D1-26	6	23 (cDNA) (full-length)	012	3

[0137] As will be observed, antibodies in accordance with the present invention were generated with a strong bias towards the utilization of the DP-50 heavy chain variable region. The DP-50 gene is also referred to as a V_H 3-33 family gene. In XenoMouse™ mice, there are more than 30 distinct functional heavy chain variable genes with which to generate antibodies. Bias, therefore, is indicative of a preferred binding motif of the antibody-antigen interaction with respect to the combined properties of binding to the antigen and functional activity.

[0138] In some embodiments, the antibody is a single-chain antibody (scFv) in which a V_L and V_H domains are paired to form a monovalent molecules via a synthetic linker that enables them to be made as a single protein chain. Bird et al., *Science* 242:423-426 (1988) and Huston et al., *Proc. Natl. Acad. Sci. USA* 85:5879-5883 (1988). In some embodiments, the antibodies are diabodies, i.e., are bivalent antibodies in which V_H and V_L domains are expressed on a single polypeptide chain, but using a linker that is too short to allow for pairing between the two domains on the same chain, thereby forcing the domains to pair with complementary domains of another chain and creating two antigen binding sites. See e.g., Holliger P. et al., *Proc. Natl. Acad. Sci. USA* 90:6444-6448 (1993), and Poljak R. J. et al., *Structure* 2:1121-1123 (1994). In some embodiments, one or more CDRs from an antibody of the invention may be incorporated into a molecule either covalently or noncovalently to make it an immunoadhesin that specifically binds to CTLA-4. In such embodiments, the CDR(s) may be incorporated as part of a larger polypeptide chain, may be covalently linked to another polypeptide chain, or may be incorporated noncovalently.

[0139] In another embodiment, the anti-CTLA-4 antibody has selectivity (or specificity) for CTLA-4 that is at least 100 times greater than its selectivity for any other polypeptide. In some embodiments, the anti-CTLA-4 antibody does not exhibit any appreciable specific binding to any other protein other than CTLA-4. One can determine the selectivity of the anti-CTLA-4 antibody for CTLA-4 using methods well known in the art following the teachings of the specification. For instance, one can determine the selectivity using Western blot, FACS, ELISA, or RIA. Thus, in some embodiments, the monoclonal anti-CTLA-4 antibody is capable of specifically binding to CTLA-4.

[0140] In some embodiments, the C-terminal lysine of the heavy chain of the anti-CTLA-4 antibody of the invention is not present. In various embodiments of the invention, the heavy and light chains of the anti-CTLA-4 antibodies may optionally include a signal sequence.

[0141] Table 3 lists the sequence identifiers (SEQ ID NOS) of the nucleic acids that encode the heavy and light chains and the corresponding predicted amino acid sequences for the anti-CTLA-4 monoclonal antibody 11.2.1.

TABLE 3

HUMAN ANTI-CTLA-4 ANTIBODY 11.2.1

	SEQUENCE IDENTIFIER (SEQ ID NOS:)			
	Heavy		Light	
MAb	cDNA	Amino Acid	cDNA	Amino Acid
11.2.1 (full-length)	21	22	23	24

[0142] In some embodiments, the nucleic acid molecule comprises a nucleotide sequence that encodes the light chain sequence of monoclonal antibody 11.2.1 (SEQ ID NO: 23), or a portion thereof. In some embodiments, the nucleic acid molecule comprises a nucleotide sequence that encodes the V_L amino acid sequence of monoclonal antibody 11.2.1 (SEQ ID NO: 26), or a portion thereof. In some embodiments, the nucleic acid molecule comprises a nucleotide sequence that encodes the light chain amino acid sequence of monoclonal antibody 11.2.1 (SEQ ID NO: 24), or a portion thereof. In some embodiments, said portion comprises at least the CDR2 region. In some embodiments, the nucleic acid encodes the amino acid sequence of the light chain CDRs of said antibody. In some embodiments, said portion is a contiguous portion comprising CDR1-CDR3. In certain aspects, the light chain CDR1 amino acid sequence is indicated by SEQ ID NO: 30, the light chain CDR2 amino acid sequence by SEQ ID NO: 31, and the light chain CDR3 amino acid sequence by SEQ ID NO: 32.

[0143] In other embodiments, the nucleic acid molecule encodes a light chain amino acid sequence that is at least 70%, 75%, 80%, 85%, 90%, 95%, 97%, 98%, 99%, or 100% identical to a light chain amino acid sequence of SEQ ID NO: 24.

[0144] In some embodiments, the nucleic acid molecule comprises a nucleotide sequence that encodes the heavy chain sequence of monoclonal antibody 11.2.1 (SEQ ID NO: 21), or a portion thereof. In some embodiments, the nucleic acid molecule comprises a nucleotide sequence that encodes the V_H amino acid sequence of monoclonal antibody 11.2.1 (SEQ ID NO: 25), or a portion thereof. In other embodiments, the nucleic acid molecule comprises a nucleotide sequence that encodes the heavy chain amino acid sequence of SEQ ID NO: 24, or a portion thereof. Nucleic acid molecules of the invention include nucleic acids that hybridize under highly stringent conditions, such as those described herein, to a nucleic acid sequence encoding the light chain amino acid sequence of SEQ ID NO: 24.

[0145] In further embodiments, the nucleic acid molecule comprises a nucleotide sequence that encodes at least a portion of the V_H amino acid sequence of 11.2.1 (SEQ ID NO: 25) or said sequence having conservative amino acid mutations and/or a total of three or fewer non-conservative amino acid substitutions. In various embodiments the sequence encodes one or more CDR regions, preferably a CDR3 region, all three CDR regions, a contiguous portion including CDR1-CDR3, or the entire V_H region. In certain aspects, the

heavy chain CDR1 amino acid sequence is indicated by SEQ ID NO: 27, the heavy chain CDR2 amino acid sequence by SEQ ID NO: 28, and the heavy chain CDR3 amino acid sequence by SEQ ID NO: 29.

[0146] In certain aspects, the present invention provides a liquid pharmaceutical composition comprising at least one isolated human antibody that binds to CTLA-4, wherein the antibody comprises a V_H amino acid sequence that utilizes a human V_H 3-33 germline gene; and a pharmaceutically acceptable excipient comprising a chelating agent.

[0147] In other aspects, the present invention provides a liquid pharmaceutical composition comprising at least one isolated human antibody that binds to CTLA-4, wherein the antibody comprises a heavy chain amino acid sequence with at least 90% sequence identity to SEQ ID NO: 22 and a light chain amino acid sequence with at least 90% sequence identity to SEQ ID NO: 24.

[0148] In other aspects, the present invention provides a liquid pharmaceutical composition comprising at least one isolated human antibody that binds to CTLA-4, wherein the antibody comprises a heavy chain amino acid sequence with at least 95% sequence identity to SEQ ID NO: 22 and a light chain amino acid sequence with at least 95% sequence identity to SEQ ID NO: 24.

[0149] In other aspects, the present invention provides a liquid pharmaceutical composition comprising at least one isolated human antibody that binds to CTLA-4, wherein the antibody comprises a heavy chain amino acid sequence with at least 99% sequence identity to SEQ ID NO: 22 and a light chain amino acid sequence with at least 99% sequence identity to SEQ ID NO: 24.

[0150] In still other aspects, the anti-CTLA-4 antibody comprises a heavy chain amino acid sequence that comprises the variable region of SEQ ID NO: 22 and a light chain amino acid sequence that comprises the variable region SEQ ID NO: 24. In further aspects, the anti-CTLA-4 antibody comprises a heavy chain variable region amino acid sequence comprising SEQ ID NO: 25 and a light chain variable region amino acid sequence comprising SEQ ID NO: 26. In further aspects, the anti-CTLA-4 antibody comprises a heavy chain amino acid sequence comprising SEQ ID NO: 22 and a light chain amino acid sequence comprising SEQ ID NO: 24. In still other aspects, the anti-CTLA-4 antibody comprises a V_H amino acid sequence comprising human FR1, FR2, and FR3 sequences that utilize a human V_H 3-33 gene family operably linked in frame with a CDR1, a CDR2, and a CDR3 sequence.

[0151] In one embodiment, the anti-CTLA-4 antibody is ticilimumab (also known as CP-675,206), which has the heavy and light chain amino acid sequences of antibody ticilimumab. In one embodiment of the present invention, the anti-CTLA-4 antibodies specifically bind to a conformational epitope on human CTLA-4. In other embodiments, the anti-CTLA-4 antibodies inhibit human tumor growth after administration to a subject.

Human IgG2 Anti-MAdCAM Antibodies:

[0152] In other embodiments, the human IgG2 antibodies that are suitable for use with the present invention include anti-MAdCAM antibodies. Suitable anti-MAdCAM antibodies and methods to prepare them that are described in International Application Number PCT/US2005/000370, filed 7 Jan. 2005 and published 28 Jul. 2005. In other embodiments, the anti-MAdCAM antibodies which are suitable for use with the present invention include those anti-MAdCAM monoclonal antibodies having the heavy and light chain amino acid sequences of the antibody designated 7.16.6 in International Application Number PCT/US2005/000370.

[0153] Suitable anti-MAdCAM antibodies for use with the present invention may be chosen from polyclonal or monoclonal antibodies. In certain aspects, the monoclonal anti-MAdCAM antibody can be a human antibody. In further embodiments, the monoclonal anti-MAdCAM antibody is a human monoclonal anti-MAdCAM antibody. The anti-MAdCAM antibody can be a human IgG2 antibody.

[0154] In addition, such anti-MAdCAM antibodies may be chosen based on differences in the amino acid sequences in the constant region of their heavy chains. For example, the anti-MAdCAM antibodies may be chosen from the IgG class, which have "gamma" type heavy chains. The class and subclass of anti-MAdCAM antibodies may be determined by any method known in the art. In general, the class and subclass of an antibody may be determined using antibodies that are specific for a particular class and subclass of antibody. Such antibodies are commercially available. The class and subclass can be determined by ELISA, or Western Blot as well as other techniques. Alternatively, the class and subclass may be determined by sequencing all or a portion of the constant domains of the heavy and/or light chains of the antibodies, comparing their amino acid sequences to the known amino acid sequences of various class and subclasses of immunoglobulins, and determining the class and subclass of the antibodies.

[0155] In some embodiments, the antibody is a single-chain antibody (scFv) in which a V_L and V_H domains are paired to form a monovalent molecules via a synthetic linker that enables them to be made as a single protein chain. (Bird et al., Science 242:423-426 (1988) and Huston et al., Proc. Natl. Acad. Sci. USA 85:5879-5883 (1988)). In some embodiments, the antibodies are diabodies, i.e., are bivalent antibodies in which V_H and V_L domains are expressed on a single polypeptide chain, but using a linker that is too short to allow for pairing between the two domains on the same chain, thereby forcing the domains to pair with complementary domains of another chain and creating two antigen binding sites. (See e.g., Holliger P. et al., Proc. Natl. Acad. Sci. USA 90:6444-6448 (1993), and Poljak R. J. et al., Structure 2:1121-1123 (1994)). In some embodiments, one or more CDRs from an antibody of the invention may be incorporated into a molecule either covalently or noncovalently to make it an immunoadhesin that specifically binds to MAdCAM. In such embodiments, the CDR(s) may be incorporated as part of a larger polypeptide chain, may be covalently linked to another polypeptide chain, or may be incorporated noncovalently.

[0156] In another embodiment, the anti-MAdCAM antibody has selectivity (or specificity) for MAdCAM that is at least 100 times greater than its selectivity for any other polypeptide. In some embodiments, the anti-MAdCAM antibody does not exhibit any appreciable specific binding to any other protein other than MAdCAM. One can determine the selectivity of the anti-MAdCAM antibody for MAdCAM using methods well known in the art following the teachings of the specification. For instance, one can determine the selectivity using Western, blot, FACS, ELISA, or RIA. Thus, in some embodiments, the monoclonal anti-MAdCAM antibody is capable of specifically binding to MAdCAM.

[0157] In some embodiments, the C-terminal lysine of the heavy chain of the anti-MAdCAM antibody of the invention

is not present. In various embodiments of the invention, the heavy and light chains of the anti-MAdCAM antibodies may optionally include a signal sequence.

[0158] Table 4 lists the sequence identifiers (SEQ ID NOS) of the nucleic acids that encode the heavy and light chains and the corresponding predicted amino acid sequences for the anti-MAdCAM monoclonal antibody 7.16.6. While DNA sequences encoding a signal polypeptide are shown in the sequence identifiers (SEQ ID NOS), the antibody typically does not comprise a signal polypeptide because the signal polypeptide is generally eliminated during post-translational modifications. In various embodiments of the invention, one or both of the heavy and light chains of the anti-MAdCAM antibodies includes a signal sequence (or a portion of the signal sequence). In other embodiments of the invention, neither the heavy nor light chain of the anti-MAdCAM antibodies includes a signal sequence.

TABLE 4

HUMAN ANTI-MAdCAM ANTIBODY 7.16.6

	SEQUENCE IDENTIFIER (SEQ ID NOS:)			
	Heavy		Light	
MAb	DNA	Amino Acid	DNA	Amino Acid
7.16.6	41	42	43	44

[0159] In some embodiments, the nucleic acid molecule comprises a nucleotide sequence that encodes the V_L amino acid sequence of monoclonal antibody 7.16.6 (SEQ ID NO: 44), or a portion thereof. In some embodiments, said portion comprises at least the CDR2 region. In some embodiments, the nucleic acid encodes the amino acid sequence of the light chain CDRs of said antibody. In some embodiments, said portion is a contiguous portion comprising CDR1-CDR3.

[0160] In still other embodiments, the nucleic acid molecule encodes a V_L amino acid sequence that is at least 70%, 75%, 80%, 85%, 90%, 95%, 96%, 97%, 98%, 99% or 100% identical to a V_L amino acid sequence of antibody 7.16.6, or an amino acid sequence of SEQ ID NO: 44. Nucleic acid molecules of the invention include nucleic acids that hybridize under highly stringent conditions, such as those described above, to a nucleic acid sequence encoding the light chain amino acid sequence of SEQ ID NO: 44.

[0161] In further embodiments, the nucleic acid molecule comprises a nucleotide sequence that encodes at least a portion of the V_H amino acid sequence of 7.16.6 (SEQ ID NO: 42) or said sequence having conservative amino acid mutations and/or a total of three or fewer non-conservative amino acid substitutions. In various embodiments the sequence encodes one or more CDR regions, preferably a CDR3 region, all three CDR regions, a contiguous portion including CDR1-CDR3, or the entire V_H region.

[0162] In some embodiments, the nucleic acid molecule encodes a V_H amino acid sequence that is at least 70%, 75%, 80%, 85%, 90%, 95%, 96%, 97%, 98%, 99% or 100% identical to the V_H amino acid sequence of SEQ ID NO: 42. Nucleic acid molecules of the invention include nucleic acids that hybridize under highly stringent conditions, such as those described above, to a nucleotide sequence encoding the heavy chain amino acid sequence of SEQ ID NO: 42.

[0163] In other aspects, the present invention provides a composition comprising at least one purified human antibody that binds to MAdCAM, wherein the antibody comprises a heavy chain amino acid sequence with at least 95% sequence identity to SEQ ID NO: 42 and a light chain amino acid sequence with at least 95% sequence identity to SEQ ID NO: 44. In other aspects, the antibody comprises a heavy chain amino acid sequence with at least 99% sequence identity to SEQ ID NO: 42 and a light chain amino acid sequence with at least 99% sequence identity to SEQ ID NO: 44. In still other aspects, the antibody comprises a heavy chain amino acid sequence that comprises the variable region of SEQ ID NO: 42 and a light chain amino acid sequence that comprises the variable region SEQ ID NO: 44. In further aspects, the antibody comprises a heavy chain amino acid sequence comprising SEQ ID NO: 42 and a light chain amino acid sequence comprising SEQ ID NO: 44.

[0164] In one embodiment of the present invention, the anti-MAdCAM antibodies specifically bind to a conformational epitope on human MAdCAM. In other embodiments, the anti-MAdCAM antibodies inhibit human tumor growth after administration to a subject.

Preparation of the Monoclonal Human IgG2 Antibody Compositions:

[0165] The human IgG2 antibody typically is formulated as a pharmaceutical composition for parenteral administration to a subject. In certain embodiments, the pharmaceutical composition is a liquid composition.

[0166] In one embodiment, the invention is directed to compositions comprising a human IgG2 antibody and a chelating agent. In another embodiment, the invention is directed to a liquid pharmaceutical composition comprising a human IgG2 antibody and a chelating agent. In another embodiment, the invention is directed to a composition comprising a human IgG2 antibody and EDTA. In another embodiment, the invention is directed to a liquid pharmaceutical composition comprising a human IgG2 antibody and EDTA.

[0167] The term "pharmaceutical composition" refers to preparations which are in such form as to permit the biological activity of the active ingredients to be effective. In some embodiments, the pharmaceutical composition is a liquid pharmaceutical composition. "Pharmaceutically acceptable excipients" (vehicles, additives) are those, which can reasonably (i.e., safely) be administered to a subject to provide an effective dose of the active ingredient employed. The term "excipient" or "carrier" as used herein refers to an inert substance, which is commonly used as a diluent, vehicle, preservative, binder or stabilizing agent for drugs. As used herein, the term "diluent" refers to a pharmaceutically acceptable (safe and non-toxic for administration to a human) solvent and is useful for the preparation of the liquid compositions herein. Exemplary diluents include, but are not limited to, sterile water and bacteriostatic water for injection (BWFI).

[0168] The compositions of the present invention involve one or more human IgG2 monoclonal antibodies of the invention in combination with pharmaceutically acceptable excipients, which comprise a chelating agent. The liquid compositions of the present invention involve one or more human IgG2 monoclonal antibodies of the invention in combination with a pharmaceutically acceptable excipient, which comprise a chelating agent. In one embodiment, the present invention encompasses a composition comprising at least one anti-

body having the heavy and light chain amino acid sequences of a human monoclonal IgG2 antibody; and a chelating agent. In another embodiment, the present invention encompasses a liquid composition comprising at least one antibody having the heavy and light chain amino acid sequences of a human monoclonal IgG2 antibody; a chelating agent; and optionally further comprising additional pharmaceutically acceptable excipients.

[0169] The concentration of the human IgG2 antibody in the compositions of the present invention is generally at least about 0.1 milligram per milliliter (mg/ml) or higher, at least about 1.0 mg/ml or higher, at least about 10 mg/ml or higher, at least about 20 mg/ml or higher, at least about 50 mg/ml or higher, at least about 100 mg/ml or higher, or at least about 200 mg/ml or higher. In certain embodiments, the concentration of the human IgG2 antibody generally ranges from about 0.1 mg/ml to about 200 mg/ml, from about 0.5 mg/ml to about 100 mg/ml, from about 1 mg/ml to about 50 mg/ml, from about 2.0 mg/ml to about 35 mg/ml, from about 5.0 mg/ml to about 25 mg/ml, or from about 7 mg/ml to about 15 mg/ml. In one embodiment, the concentration of the human IgG2 antibody in the compositions of the present invention is generally about 5 mg/ml, about 10 mg/ml, about 20 mg/ml, about 50 mg/ml, about 65 mg/ml, about 70 mg/ml, about 75 mg/ml, about 80 mg/ml, about 85 mg/ml, or about 100 mg/ml. In another embodiment, the concentration of the human IgG2 antibody in the composition ranges from about 1 mg/ml to about 50 mg/ml. In one embodiment, the concentration of the human IgG2 antibody in the composition is about 10 mg/ml. In another embodiment, the concentration of the human IgG2 antibody is about 75 mg/ml.

[0170] In another embodiment, the concentration of the human IgG2 antibody in the compositions ranges from about 50 mg/ml to about 100 mg/ml. In some embodiments, higher antibody concentrations can be used where the composition is intended for subcutaneous delivery.

[0171] As used herein, the terms "chelating agent" generally refers to an excipient that can form at least one bond (e.g., covalent, ionic, or otherwise) to a metal ion. A chelating agent is typically a multidentate ligand that can be used in selected liquid compositions as a stabilizer to complex with species, which might promote instability. Often, compounds that can act as a chelating agent will have electron-rich functional groups. Suitable electron-rich functional groups include carboxylic acid groups, hydroxy groups and amino groups. Arrangement of these groups in aminopolycarboxylic acids, hydroxypolycarboxylic acids, hydroxyaminocarboxylic acids, and the like, result in moieties that have the capacity to bind metal.

[0172] However, the present invention is not intended to be limited to chelating agents primarily by the chelating agent's ability to form bonds with a metal ion. Therefore, the present invention is not intended to be limited by any specific mechanism by which the chelating agent acts in the compositions of the present invention and the excipients termed chelating agents herein may achieve their properties through mechanisms that are altogether unrelated to the chelating agent's ability to form bonds with a metal ion.

[0173] Chelating agents that are suitable for use in the present invention, include, but are not limited to, aminopolycarboxylic acids, hydroxyaminocarboxylic acids, N-substituted glycines, 2-(2-amino-2-oxoethyl)aminoethane sulfonic acid (BES), deferoxamine (DEF), citric acid, niacinamide, and desoxycholates. Examples of suitable aminopolycarboxylic acids include ethylenediaminetetraacetic acid (EDTA), diethylenetriamine pentaacetic acid 5 (DTPA), nitrilotriacetic acid (NTA), N-2-acetamido-2-iminodiacetic acid (ADA), bis(aminoethyl)glycolether, N,N,N',N'-tetraacetic acid (EGTA), trans-diaminocyclohexane tetraacetic acid (DCTA), glutamic acid, and aspartic acid. Examples of suitable hydroxyaminocarboxylic acids include N-hydroxyethyliminodiacetic acid (HIMDA); N,N-bis-hydroxyethylglycine (bicine) and N-(trishydroxymethylmethyl) 10 glycine (tricine). An example of a suitable N-substituted glycine is glycylglycine. An example of a suitable desoxycholate is sodium desoxycholate. Mixtures of two or more chelating agents are also encompassed by the present invention.

[0174] Chelating agents used in the invention can be present, where possible, as the free acid or free base form of the compound (e.g., referred to interchangeably herein as "EDTA" or "edetate") or as a corresponding salt form (e.g., the corresponding acid addition salt or base addition salt, such as disodium edetate). Suitable acid addition salts, e.g., include alkali metal salts (e.g., sodium or potassium salts), alkaline earth metal salts (e.g., calcium salts), and salts can be prepared using other weakly bound metal ions. As is known in the art, the nature of the salt and the number of charges to be neutralized will depend on the number of carboxyl groups present and the pH at which the stabilizing chelating agent is supplied. As is also known in the art, chelating agents have varying strengths with which particular target ions are bound. By way of further illustration, suitable salts of EDTA include dipotassium edetate, disodium edetate, edetate calcium disodium, sodium edetate, trisodium edetate, and potassium edetate; and a suitable salt of deferoxamine (DEF) is deferoxamine mesylate (DFM).

[0175] Chelating agents used in the invention can be present as an anhydrous, solvated or hydrated form of the compound or corresponding salt. Where the chelating agent is in a solvated or hydrated form, it can be present in varying states of solvation or hydration (including, e.g., anhydrous, hydrated, dihydrated, and trihydrated forms). By way of further illustration, a suitable hydrate of EDTA is disodium EDTA dihydrate; and suitable forms of citric acid include anhydrous citric acid, citric acid monohydrate, and trisodium citrate-dihydrate.

[0176] Suitable chelating agents used in the antibody compositions of the present invention also include, for example, those that bind to metal ions in solution to render them unable to react with available O_2, thereby minimizing or preventing generation of hydroxyl radicals which are free to react with and degrade the antibody. Chelating agents can lower the formation of reduced oxygen species, reduce acidic species (e.g., deamidation) formation, reduce antibody aggregation, and/or reduce antibody fragmentation in the compositions of the present invention. Such chelating agents can reduce or prevent degradation of an antibody that is formulated without the protection of a chelating agent.

[0177] When a concentration of a chelating agent is referred to, it is intended that the recited concentration represent the molar concentration of the free acid or free base form of the chelating agent. For example, the concentration of chelating agent in certain liquid pharmaceutical compositions generally ranges from about 0.01 micromolar to about 50 millimolar, from about 1 micromolar to about 10.0 millimolar, from about 15 micromolar to about 5.0 millimolar, from about 0.01 millimolar to about 1.0 millimolar, or from about 0.03 millimolar to about 0.5 millimolar. In certain embodi-

ments, the concentration of chelating agent in the liquid pharmaceutical composition can be about 0.01 millimolar, 0.02 millimolar, 0.027 millimolar, 0.03 millimolar, about 0.04 millimolar, about 0.05 millimolar, about 0.06 millimolar, about 0.07 millimolar, about 0.10 millimolar, about 0.20 millimolar, about 0.26 millimolar, about 0.27 millimolar, about 0.30 millimolar, about 0.31 millimolar, about 0.34 millimolar, about 0.40 millimolar, about 0.50 millimolar, or about 1.0 millimolar. In certain embodiments, the concentration of chelating agent is about 0.027 millimolar, about 0.05 millimolar, about 0.13 millimolar, or about 0.27 millimolar. In one embodiment, the concentration of chelating agent is about 0.05 millimolar. In another embodiment, the concentration of chelating agent is about 0.13 millimolar.

[0178] Unless stated otherwise, the concentrations listed herein are those concentrations at ambient conditions, (i.e., at 25° C. and atmospheric pressure). Ranges intermediate to the above-recited chelating agent concentrations are also intended to be part of this invention. For example, ranges of values using a combination of any of the above-recited values as upper and/or lower limits are intended to be included.

[0179] In one embodiment, the chelating agent is selected from the group consisting of EDTA, DTPA, DFM, and mixtures thereof. In another embodiment, the chelating is agent is DFM. In another embodiment, the chelating agent is EDTA. In another embodiment, the chelating agent is DTPA. In another embodiment, the composition comprises EDTA in an amount that generally ranges from about 0.01 micromolar to about 50 millimolar, from about 1 micromolar to about 20.0 millimolar, from about 15 micromolar to about 10.0 millimolar, from about 0.01 millimolar to about 5.0 millimolar, or from about 0.03 millimolar to about 1 millimolar. In certain embodiments, the concentration of EDTA in the composition can be about 0.01 millimolar, 0.02 millimolar, 0.027 millimolar, 0.03 millimolar, about 0.04 millimolar, about 0.05 millimolar, about 0.06 millimolar, about 0.07 millimolar, about 0.10 millimolar, about 0.20 millimolar, about 0.26 millimolar, about 0.27 millimolar, about 0.30 millimolar, about 0.31 millimolar, about 0.34 millimolar, about 0.40 millimolar, about 0.50 millimolar, or about 1.0 millimolar. In certain embodiments, the concentration of EDTA is about 0.027 millimolar, about 0.05 millimolar, about 0.13 millimolar, or about 0.27 millimolar. In one embodiment, the concentration of EDTA is about 0.05 millimolar. In another embodiment, the concentration of EDTA is about 0.13 millimolar.

[0180] As noted above, the compositions of the present invention optionally may further comprise a buffer in addition to a chelating agent. As used herein, the term "buffer" refers to an added composition that allows a liquid antibody composition to resist changes in pH.

[0181] In certain embodiments, the added buffer allows a composition (when in liquid form) to resist changes in pH by the action of its acid-base conjugate components. For example, a buffered composition may be prepared by adding L-histidine-HCl (L-histidine-hydrochloride) and L-histidine in the appropriate amounts to arrive at a desired pH. However, in other embodiments, the added buffer allows a liquid antibody composition to resist changes in pH by the action of its acid-base conjugate components. By way of a second example, a buffered composition may be prepared by adding an acid, such as hydrochloric acid, and L-histidine in the appropriate amounts to arrive at a desired pH.

[0182] Examples of suitable buffers include, but are not limited to, acetate (e.g., sodium acetate), succinate (e.g., sodium succinate), gluconate, citrate (e.g., and other organic acid buffers, including, but not limited to, buffers such as amino acids (e.g., histidine), acetic acid, phosphoric acid and phosphates, ascorbate, tartartic acid, maleic acid, glycine, lactate, lactic acid, ascorbic acid, imidazoles, carbonic acid and bicarbonates, succinic acid, sodium benzoic acid and benzoates, gluconate, edetate (EDTA), acetate, malate, imidazole, tris, phosphate, and mixtures thereof. In one embodiment, the buffer is acetate.

[0183] In another embodiment, the buffer is histidine. The histidine starting material used to prepare the compositions of the present invention can exist in different forms. For example, the histidine can be an enantiomeric (e.g., L- or D-enantiomer) or racemic form of histidine, a free acid or free base form of histidine, a salt form (e.g., a monohydrochloride, dihydrochloride, hydrobromide, sulfate, or acetate salt) of histidine, a solvated form of histidine, a hydrated form (e.g., monohydrate) of histidine, or an anhydrous form of histidine. The purity of histidine base and/or salt used to prepare the compositions generally can be at least about 98%, at least about 99%, or at least about 99.5%. As used herein, the term "purity" in the context of histidine refers to chemical purity of histidine as understood in the art, e.g., as described in The Merck Index, 13th ed., O'Neil et al. ed. (Merck & Co., 2001).

[0184] When a concentration of a buffer is referred to, it is intended that the recited concentration represent the molar concentration of the free acid or free base form of the buffer. For example, the concentration of the buffer when present in certain liquid pharmaceutical compositions can range from about 0.1 millimolar (mM) to about 100 mM. In one embodiment, the concentration of the buffer is from about 1 mM to about 50 mM. In another embodiment, the concentration of the buffer is from about 5 mM to about 30 mM. In various embodiments, the concentration of the buffer is about 1 mM, about 5 mM, about 10 mM, about 15 mM, about 20 mM, about 25 mM, about 30 mM, about 35 mM, about 40 mM, about 45 mM, about 50 mM, about 55 mM, about 60 mM, about 65 mM, about 70 mM, about 75 mM, about 80 mM, about 85 mM, about 90 mM, about 95 mM or about 100 mM. In one embodiment, the concentration of histidine in the pharmaceutical composition is about 10 mM. In another embodiment, the pharmaceutical composition contains about 10 mM of L-histidine (in base form). In another embodiment, the concentration of histidine in the pharmaceutical composition is about 20 mM. In another embodiment, the pharmaceutical composition contains, about 20 mM of L-histidine (in base form). Ranges intermediate to the above-recited histidine concentrations are also intended to be part of this invention. For example, ranges of values using a combination of any of the above-recited values as upper and/or lower limits are intended to be included.

[0185] In general, the buffer is used to maintain an acceptable pH level (which can affect antibody stability) in the compositions. The composition are typically buffered to maintain a pH in the range of from about 4 to about 8; from about 4.5 to about 7; from about 5.0 to 6.5, from about 5.2 to about 5.8, or from about 5.2 to about 6.3. Ranges intermediate to the above-recited pH's are also intended to be part of this invention. For example, ranges of values using a combination of any of the above-recited values as upper and/or lower limits are intended to be included. In one embodiment, the composition is buffered to maintain a pH of about 5.5. In another embodiment, the composition is buffered to maintain a pH of about 6.0.

[0186] As noted above, the compositions of the present invention optionally may further comprise a pharmaceutically acceptable tonicity agent in addition to a chelating agent. As used herein, the terms "tonicity agent" or "tonicifier" refers to an excipient that can adjust the osmotic pressure of a liquid antibody composition. In certain embodiments, the tonicity agent can adjust the osmotic pressure of a liquid antibody composition to isotonic so that the antibody composition is physiologically compatible with the cells of the body tissue of the subject. In still other embodiments, the "tonicity agent" may contribute to an improvement in stability of any of the human IgG2 antibodies described herein. An "isotonic" composition is one that has essentially the same osmotic pressure as human blood. Isotonic compositions generally have an osmotic pressure from about 250 to 350 mOsm. The term "hypotonic" describes a composition with an osmotic pressure below that of human blood. Correspondingly, the term "hypertonic" is used to describe a composition with an osmotic pressure above that of human blood. Isotonicity can be measured using a vapor pressure or ice-freezing type osmometer, for example.

[0187] The tonicity agent used to prepare the compositions of the present invention can exist in different forms. When the tonicity agent is referred to, it is intended that all of these different forms are encompassed by the name of the tonicity agent. For example, the tonicity agent can be in an enantiomeric (e.g., L- or D-enantiomer) or racemic form; isomers such as alpha or beta, including alpha, alpha; or beta, beta; or alpha, beta; or beta, alpha; a free acid or free base form; a hydrated form (e.g., monohydrate), or an anhydrous form.

[0188] In one embodiment, the tonicity agent is a saccharide. As used herein, the term "saccharide" refers to a class of molecules that are derivatives of polyhydric alcohols. Saccharides are commonly referred to as carbohydrates and may contain different amounts of sugar (saccharide) units, e.g., monosaccharides, disaccharides and polysaccharides. Saccharides that are suitable for use as a tonicity agent in the present invention, include, but are not limited to, saccharides selected from the group consisting of fructose, glucose, mannose, sorbose, xylose, lactose, maltose, sucrose, dextran, pullulan, dextrin, cyclodextrins, soluble starch, hydroxyethyl starch, water-soluble glucans, and mixtures thereof.

[0189] In another embodiment, the tonicity agent is a polyol. As used herein, the term "polyol" refers an excipient with multiple hydroxyl groups, and includes sugars (reducing and nonreducing sugars), sugar alcohols and sugar acids. In one embodiment, the polyol has a molecular weight that is less than about 600 kD (e.g., in the range from about 120 to about 400 kD). A "reducing sugar" is one which contains a hemiacetal group that can reduce metal ions or react covalently with lysine and other amino groups in proteins and a "nonreducing sugar" is one which does not have these properties of a reducing sugar. Polyols that are suitable for use as a tonicity agent in the present invention, include, but are not limited to, polyols selected from the group consisting of mannitol, trehalose, sorbitol, erythritol, isomalt, lactitol, maltitol, xylitol, glycerol, lactitol, propylene glycol, polyethylene glycol, inositol, and mixtures thereof. In one embodiment, the tonicity agent is a non-reducing sugar selected from the group consisting of trehalose, sucrose, and mixtures thereof. In one embodiment, the tonicity agent is a salt, such as sodium chloride.

[0190] In one embodiment, the tonicity agent is mannitol. In another embodiment, the tonicity agent is D-mannitol. In another embodiment, the tonicity agent is trehalose. In another embodiment, the tonicity agent is a α-trehalose dihydrate. In another embodiment, the tonicity agent is sucrose. In another embodiment, the tonicity agent is sodium chloride.

[0191] In one embodiment, concentration of the tonicity agent in the composition ranges from about 1 millimolar to about 600 millimolar, from about 1 millimolar to about 400 millimolar, from 1 millimolar to about 300 millimolar, or from 200 millimolar to about 275 millimolar. In one another embodiment, the tonicity agent is mannitol and is present in the liquid pharmaceutical composition at a concentration of about 247 millimolar. In another embodiment, the tonicity agent is trehalose and is present in the composition at a concentration of about 222 millimolar. In another embodiment, the tonicity agent is trehalose and is present in the composition at a concentration of about 238 millimolar. In another embodiment, the tonicity agent is sucrose is present in the composition at a concentration of about 263 millimolar.

[0192] In one embodiment, concentration of the tonicity agent in the composition ranges from about 1 mg/ml to about 300 mg/ml, from about 1 mg/ml to about 200 mg/ml, or from about 50 mg/ml to about 150 mg/ml. In another embodiment, the tonicity agent is mannitol and is present in the composition at a concentration of about 45 mg/ml. In another embodiment, the tonicity agent is trehalose and is present in the composition at a concentration of about 84 mg/ml. In another embodiment, the tonicity agent is trehalose and is present in the composition at a concentration of about 90 mg/ml. In another embodiment, the tonicity agent is trehalose and is present in the composition at a concentration of about 104 mg/ml. In another embodiment, the tonicity agent is sucrose and is present in the composition at a concentration of about 90 mg/ml. In another embodiment, the tonicity agent is sucrose and is present in the composition at a concentration of about 94 mg/ml.

[0193] In one embodiment, when the tonicity agent is a salt, the concentration of the salt in the composition ranges from about 1 mg/ml to about 20 mg/ml. In another embodiment, the tonicity agent is sodium chloride and the concentration of the sodium chloride in the composition is about 8.18 mg/ml.

[0194] Ranges intermediate to the above-recited tonicity agent concentrations are also intended to be part of this invention. For example, ranges of values using a combination of any of the above-recited values as upper and/or lower limits are intended to be included.

[0195] As noted above, the compositions of the present invention optionally may further comprise a pharmaceutically acceptable surfactant in addition to a chelating agent. As used herein, the term "surfactant" refers to an excipient that can alter the surface tension of a liquid antibody composition. In certain embodiments, the surfactant reduces the surface tension of a liquid antibody composition. In still other embodiments, the "surfactant" may contribute to an improvement in stability of any of the human IgG2 antibodies described herein. For example, the surfactant may reduce aggregation of the formulated antibody and/or minimize the formation of particulates in the composition and/or reduces adsorption. The surfactant may also improve stability of the antibody during and after a freeze/thaw cycle.

[0196] Suitable surfactants include polysorbate surfactants, poloxamers (e.g., poloxamer 18 and 407), triton surfactants such as Triton X-100®, polysorbate surfactants such as Tween 20® and Tween 800), sodium dodecyl sulfate, sodium laurel sulfate, sodium octyl glycoside, lauryl-sulfobetaine,

myristyl-sulfobetaine, linoleyl-sulfobetaine, stearyl-sulfobetaine, lauryl-sarcosine, myristyl-sarcosine, linoleyl-sarcosine, stearyl-sarcosine, linoleyl-betaine, myristyl-betaine, cetyl-betaine, lauroamidopropyl-betaine, cocamidopropyl-betaine, linoleamidopropyl-betaine, myristamidopropyl-betaine, palmidopropyl-betaine, isostearamidopropyl-betaine, myristamidopropyl-dimethylamine, palmidopropyl-dimethylamine, isostearamidopropyl-dimethylamine, sodium methyl cocoyl-taurate, disodium methyl oleyl-taurate, dihydroxypropyl PEG 5 linoleammonium chloride, polyethylene glycol, polypropylene glycol, and mixtures thereof.

[0197] In one embodiment, the surfactant is a polysorbate surfactant comprising at least one excipient that is selected from the group consisting of polysorbate 20, polysorbate 21, polysorbate 40, polysorbate 60, polysorbate 61, polysorbate 65, polysorbate 80, polysorbate 81, polysorbate 85, and mixtures thereof. In another embodiment, the composition comprises polysorbate 80.

[0198] The concentration of the surfactant when present in the composition generally ranges from about 0.01 mg/ml to about 10 mg/ml, from about 0.05 mg/ml to about 5.0 mg/ml, from about 0.1 mg/ml to about 1.0 mg/ml, or from about 0.2 mg/ml to about 0.7 mg/ml. In another embodiment, the concentration of the surfactant ranges from about 0.05 millimolar to about 1.0 millimolar. In another embodiment, the surfactant is present in an amount that is about 0.2 mg/ml. In another embodiment, the surfactant is present in an amount that is about 0.4 mg/ml.

[0199] In another embodiment, the surfactant is present in an amount that is about 0.5 mg/ml. In one embodiment, the composition contains about 0.2 mg/ml polysorbate 80. In another embodiment, the composition contains about 0.4 mg/ml polysorbate 80. In another embodiment, the composition contains about 0.5 mg/ml polysorbate 80.

[0200] Ranges intermediate to the above-recited surfactant concentrations are also intended to be part of this invention. For example, ranges of values using a combination of any of the above-recited values as upper and/or lower limits are intended to be included.

[0201] As noted above, the compositions of the present invention optionally may further comprise a pharmaceutically acceptable antioxidant in addition to a chelating agent. Suitable antioxidants include, but are not limited to, methionine, sodium thiosulfate, catalase, and platinum. For example, the composition may contain methionine in a concentration that ranges from 1 mM to about 100 mM, and in particular, is about 27 mM.

[0202] In one embodiment, the present invention encompasses a composition comprising at least one human IgG2 antibody and a chelating agent.

[0203] In one embodiment, the present invention encompasses a liquid pharmaceutical composition comprising at least one human IgG2 antibody and a pharmaceutically acceptable chelating agent.

[0204] In one embodiment, the present invention encompasses a liquid pharmaceutical composition comprising at least one human IgG2 antibody; a pharmaceutically acceptable chelating agent; and optionally including additional pharmaceutically acceptable excipients.

[0205] In one embodiment, the present invention encompasses a composition comprising at least one human IgG2 antibody and a chelating agent, wherein the antibody has a purity of at least about 90%, 95% or 100%.

[0206] In one embodiment, the present invention encompasses a composition comprising at least one human IgG2 antibody and a chelating agent, wherein the composition has an antibody concentration of from about 0.1 mg/ml to about 200 mg/ml.

[0207] In one embodiment, the present invention encompasses a composition comprising at least one human IgG2 antibody and a chelating agent, wherein the composition contains a concentration of antibody that is at least about 5 mg/ml, at least about 10 mg/ml, at least about 15 mg/ml, at least about 20 mg/ml, or at least about 25 mg/ml.

[0208] In one embodiment, the present invention encompasses a composition comprising at least one human IgG2 antibody and a chelating agent, wherein the composition contains a concentration of antibody that is at least about 60 mg/ml, at least about 70 mg/ml, at least about 75 mg/ml, at least about 80 mg/ml, or at least about 90 mg/ml.

[0209] In one embodiment, the present invention encompasses a composition comprising at least one human IgG2 antibody and from about 0.001 millimolar to about 5.0 millimolar of a chelating agent, wherein the composition has an antibody concentration of from about 0.1 mg/ml to about 200 mg/ml.

[0210] In one embodiment, the present invention encompasses a liquid pharmaceutical composition comprising at least one human IgG2 antibody and from about 0.001 millimolar to about 1.0 millimolar of a chelating agent, wherein the composition has an antibody concentration of from about 0.1 mg/ml to about 200 mg/ml.

[0211] In one embodiment, the present invention encompasses a liquid pharmaceutical composition comprising at least one human IgG2 antibody and from about 0.001 millimolar to about 0.5 millimolar of a chelating agent, wherein the composition has an antibody concentration of from about 0.1 mg/ml to about 200 mg/ml.

[0212] In one embodiment, the present invention encompasses a liquid pharmaceutical composition comprising at least one human IgG2 antibody and from about 0.01 millimolar to about 0.5 millimolar of EDTA, wherein the composition has an antibody concentration of from about 0.1 mg/ml to about 200 mg/ml.

[0213] In other aspects of the present invention, the composition comprises at least one monoclonal human IgG2 antibody; a chelating agent and a buffer.

[0214] In other aspects of the present invention, the composition comprises at least one monoclonal human IgG2 antibody; a chelating agent; a buffer; and a tonicity agent.

[0215] In other aspects of the present invention, the composition comprises at least one monoclonal human IgG2 antibody; a chelating agent; a buffer; and a tonicity agent that comprises sucrose.

[0216] In other aspects of the present invention, the composition comprises at least one monoclonal human IgG2 antibody; a chelating agent; a buffer; and a tonicity agent that comprises mannitol.

[0217] In other aspects of the present invention, the composition comprises at least one monoclonal human IgG2 antibody; a chelating agent; a buffer; and a tonicity agent that comprises trehalose.

[0218] In other aspects of the present invention, the composition comprises at least one monoclonal human IgG2 antibody; a chelating agent; a buffer; and a tonicity agent that comprises sodium chloride.

[0219] In other aspects of the present invention, the composition comprises at least one monoclonal human IgG2 antibody; a chelating agent; a buffer; a tonicity agent; and a surfactant.

[0220] In other aspects of the present invention, the composition comprises at least one monoclonal human IgG2 antibody; EDTA; and a buffer.

[0221] In other aspects of the present invention, the composition comprises at least one monoclonal human IgG2 antibody; EDTA; and acetate.

[0222] In other aspects of the present invention, the composition comprises at least one monoclonal human IgG2 antibody; EDTA; and histidine.

[0223] In other aspects of the present invention, the composition comprises at least one monoclonal human IgG2 antibody; EDTA; a buffer; and a tonicity agent.

[0224] In other aspects of the present invention, the composition comprises at least one monoclonal human IgG2 antibody; EDTA; a buffer; a tonicity agent; and a surfactant.

[0225] In other aspects of the present invention, the composition comprises at least one monoclonal human IgG2 antibody; EDTA; histidine; a tonicity agent; and a surfactant.

[0226] In other aspects of the present invention, the composition comprises at least one monoclonal human IgG2 antibody; EDTA; histidine; trehalose; and a surfactant.

[0227] In other aspects of the present invention, the composition comprises at least one monoclonal human IgG2 antibody; a chelating agent; histidine; trehalose; and polysorbate 80.

[0228] In other aspects of the present invention, the composition comprises at least one monoclonal human IgG2 antibody; EDTA; histidine; trehalose; and polysorbate 80.

[0229] In other aspects of the present invention, the composition comprises at least one monoclonal human IgG2 antibody; EDTA; histidine; sucrose; and polysorbate 80.

[0230] In other aspects of the present invention, the composition comprises at least one monoclonal human IgG2 antibody; EDTA; histidine; mannitol; and polysorbate 80.

[0231] In other aspects of the present invention, the composition comprises at least one monoclonal human IgG2 antibody; EDTA; histidine; sodium chloride; and polysorbate 80.

[0232] In one embodiment, the present invention encompasses a composition comprising at least one human IgG2 antibody; from about 0.01 millimolar to about 1.0 millimolar of a chelating agent; and from about 1 millimolar to about 100 millimolar of a buffer, wherein the composition has an antibody concentration of from about 0.1 mg/ml to about 200 mg/ml.

[0233] In one embodiment, the present invention encompasses a composition comprising at least one human IgG2 antibody; from about 0.01 millimolar to about 1.0 millimolar of a chelating agent; and from about 1 millimolar to about 50 millimolar of histidine, wherein the composition has an antibody concentration of from about 0.1 mg/ml to about 200 mg/ml.

[0234] In one embodiment, the present invention encompasses a composition comprising at least one human IgG2 antibody; from about 0.01 millimolar to about 1.0 millimolar of EDTA; and from about 1 millimolar to about 50 millimolar of histidine, wherein the composition has an antibody concentration of from about 0.1 mg/ml to about 200 mg/ml.

[0235] In one embodiment, the present invention encompasses a composition comprising at least one human IgG2 antibody; from about 0.01 millimolar to about 1.0 millimolar of a chelating agent; from about 1 millimolar to about 50 millimolar of a buffer; and from about 100 millimolar to about 600 millimolar of a tonicity agent, wherein the composition has an antibody concentration of from about 0.1 mg/ml to about 200 mg/ml.

[0236] In one embodiment, the present invention encompasses a composition comprising at least one human IgG2 antibody; from about 0.01 millimolar to about 1.0 millimolar of a chelating agent; from about 1 millimolar to about 50 millimolar of histidine; and from about 100 millimolar to about 600 millimolar of a tonicity agent, wherein the composition has an antibody concentration of from about 0.1 mg/ml to about 200 mg/ml.

[0237] In one embodiment, the present invention encompasses a composition comprising at least one human IgG2 antibody; from about 0.01 millimolar to about 1.0 millimolar of a chelating agent; from about 1 millimolar to about 50 millimolar of a buffer; from about 100 millimolar to about 600 millimolar of a tonicity agent; and from about 0.005 millimolar to about 10 millimolar of a surfactant, wherein the composition has an antibody concentration of from about 0.1 mg/ml to about 200 mg/ml.

[0238] In one embodiment, the present invention encompasses a composition comprising at least one human IgG2 antibody; from about 0.01 millimolar to about 1.0 millimolar of EDTA; from about 1 millimolar to about 50 millimolar of a buffer; from about 100 millimolar to about 600 millimolar of a tonicity agent; and from about 0.005 millimolar to about 10 millimolar of a surfactant, wherein the composition has an antibody concentration of from about 0.1 mg/ml to about 200 mg/ml.

[0239] In one embodiment, the present invention encompasses a composition comprising at least one human IgG2 antibody; from about 0.01 millimolar to about 1.0 millimolar of a chelating agent; from about 1 millimolar to about 50 millimolar of histidine; from about 100 millimolar to about 600 millimolar of trehalose; and from about 0.005 millimolar to about 10 millimolar of polysorbate 80, wherein the composition has an antibody concentration of from about 0.1 mg/ml to about 200 mg/ml.

[0240] In one embodiment, the present invention encompasses a composition comprising at least one human IgG2 antibody; from about 0.01 millimolar to about 1.0 millimolar of EDTA; from about 1 millimolar to about 50 millimolar of histidine; from about 100 millimolar to about 600, millimolar of trehalose; and from about 0.005 millimolar to about 10 millimolar of polysorbate 80, wherein the composition has an antibody concentration of from about 0.1 mg/ml to about 200 mg/ml.

[0241] In one embodiment, the composition comprises from about 0.01 mg/ml to about 200 mg/ml of at least one monoclonal human IgG2 antibody; and from about 1 mM to about 100 mM of histidine.

[0242] In other aspects of the present invention, the liquid pharmaceutical compositions comprise from about 1 mg/ml to about 100 mg/ml of a monoclonal human IgG2 antibody; from about 10 mM to about 30 mM of histidine; from about 0.05 millimolar to about 0.5 millimolar of polysorbate 80; from about 0.01 millimolar to about 1 millimolar of EDTA; and from about 200 millimolar to about 400 millimolar of trehalose.

[0243] In other aspects of the present invention, the liquid pharmaceutical compositions comprise from about 1 mg/ml to about 100 mg/ml of a monoclonal human IgG2 antibody;

from about 10 mM to about 30 mM of histidine; from about 0.05 millimolar to about 0.5 millimolar of polysorbate 80; from about 0.01 millimolar to about 1 millimolar of EDTA; and from about 200 millimolar to about 300 millimolar of trehalose.

[0244] In another embodiment, the invention is directed to a composition comprising at least one monoclonal human IgG2 antibody and DTPA.

[0245] In certain aspects of the present invention, the liquid human IgG2 antibody compositions comprise from about 1 mg/ml to about 100 mg/ml of at least one monoclonal human IgG2 antibody; from about 1 mM to about 50 mM of histidine; from about 0.01 mg/ml to about 5 mg/ml of polysorbate 80; from about 0.001 mg/ml to about 0.5 mg/ml of EDTA; and from about 10 mg/ml to about 200 mg/ml of sucrose.

[0246] In other aspects of the present invention, the human IgG2 antibody compositions comprise from about 50 mg/ml to about 100 mg/ml of at least one monoclonal human IgG2 antibody; from about 10 mM to about 30 mM of histidine; from about 0.05 millimolar to about 1 millimolar of polysorbate 80; from about 0.01 millimolar to about 1 millimolar of EDTA; and from about 100 millimolar to about 400 millimolar of sucrose.

[0247] In another embodiment, the invention is directed to a composition comprising at least one human IgG2 antibody and a chelating agent, wherein the molar concentration of the antibody ranges from about 0.01 millimolar to about 2 millimolar and the molar concentration of the chelating agent ranges from about 0.001 millimolar to about 5 millimolar, and wherein the molar ratio of antibody to chelating agent ranges from about 0.002 to about 2000; from about 0.01 to about 500; from about 0.05 to about 100; from about 0.1 to about 50; from about 0.5 to about 10; from about 1 to about 5; from about 1 to about 3; or about 1.6.

[0248] In another embodiment, the invention is directed to a composition comprising at least one human IgG2 antibody and a chelating agent, wherein the molar concentration of the antibody ranges from about 0.01 millimolar to about 2 millimolar and the molar concentration of the chelating agent ranges from about 0.001 millimolar to about 5 millimolar, and wherein the molar ratio of antibody to chelating agent ranges from about 0.002 to about 2000; from about 0.01 to about 500; from about 0.05 to about 100; from about 0.1 to about 50; from about 0.5 to about 10; from about 1 to about 5; or about 3.8.

[0249] In another embodiment, the invention is directed to a composition comprising at least one monoclonal human IgG2, a chelating agent, and histidine; wherein the molar concentration of the antibody ranges from about 0.01 millimolar to about 2 millimolar and the molar concentration of histidine ranges from about 1 millimolar to about 100 millimolar and the molar concentration of the chelating agent ranges from about 0.001 millimolar to about 5 millimolar, and wherein the molar ratio of antibody to chelating agent ranges from about 0.002 to about 2000; from about 0.01 to about 500; from about 0.05 to about 100; from about 0.1 to about 50; from about 0.5 to about 10; from about 1 to about 5; or about 3.8.

[0250] In another embodiment, the invention is directed to a composition comprising at least one monoclonal human IgG2, a chelating agent, and histidine; wherein the molar concentration of the antibody ranges from about 0.01 millimolar to about 2 millimolar and the molar concentration of histidine ranges from about 5 millimolar to about 30 millimolar and the molar concentration of the chelating agent ranges from about 0.001 millimolar to about 5 millimolar, and wherein the molar ratio of antibody to chelating agent ranges from about 0.002 to about 2000; from about 0.01 to about 500; from about 0.05 to about 100; from about 0.1 to about 50; from about 0.5 to about 10; from about 1 to about 5; or about 3.8.

[0251] In another embodiment, the invention is directed to a composition comprising at least one monoclonal human IgG2 antibody and a pharmaceutically acceptable chelating agent, wherein the molar concentration of the antibody ranges from about 0.0006 millimolar to about 1.35 millimolar and the molar concentration of the chelating agent ranges from about 0.003 millimolar to about 50 millimolar, and wherein the molar ratio of antibody to chelating agent ranges from about 0.00001 to about 450; from about 0.0001 to about 100; from about 0.005 to about 50; from about 0.001 to about 10; from about 0.01 to about 5; from about 0.1 to about 1; or is about 0.5.

[0252] In another embodiment, the invention is directed to a composition comprising at least one monoclonal human IgG2 antibody, a pharmaceutically acceptable chelating agent, and histidine; wherein the molar concentration of the antibody ranges from about 0.0006 millimolar to about 1.35 millimolar, the molar concentration of the chelating agent ranges from about 0.003 millimolar to about 50 millimolar, and the molar concentration of histidine ranges from about 1 millimolar to about 100 millimolar; and wherein the molar ratio of antibody to chelating agent ranges from about 0.00001 to about 450; from about 0.0001 to about 100; from about 0.005 to about 50; from about 0.001 to about 10; from about 0.01 to about 5; from about 0.1 to about 1; or is about 0.5.

[0253] In another embodiment, the invention is directed to a composition comprising a human IgG2 antibody and a pharmaceutically acceptable chelating agent, wherein the molar concentration of the antibody ranges from about 0.0006 millimolar to about 1.35 millimolar and the molar concentration of the chelating agent ranges from about 0.003 millimolar to about 50 millimolar, and wherein the molar ratio of antibody to chelating agent ranges from about 0.00001 to about 450; from about 0.0001 to about 100; from about 0.005 to about 50; from about 0.001 to about 10; from about 0.01 to about 5; from about 0.1 to about 3; or is about 1.9.

[0254] In another embodiment, the invention is directed to a composition comprising a human IgG2 antibody, a pharmaceutically acceptable chelating agent, and histidine; wherein the molar concentration of the antibody ranges from about 0.0006 millimolar to about 1.35 millimolar, the molar concentration of the chelating agent ranges from about 0.003 millimolar to about 50 millimolar, and the molar concentration of histidine ranges from about 1 millimolar to about 100 millimolar; and wherein the molar ratio of antibody to chelating agent ranges from about 0.00001 to about 450; from about 0.0001 to about 100; from about 0.005 to about 50; from about 0.001 to about 10; from about 0.01 to about 5; from about 0.1 to about 1; or is about 0.5.

[0255] In another embodiment, the invention is directed to a composition comprising a human IgG2 antibody, a pharmaceutically acceptable chelating agent, and histidine; wherein the molar concentration of the antibody ranges from about 0.0006 millimolar to about 1.35 millimolar, the molar concentration of the chelating agent ranges from about 0.003

millimolar to about 50 millimolar, and the molar concentration of histidine ranges from about 5 millimolar to about 30 millimolar; and wherein the molar ratio of antibody to chelating agent ranges from about 0.0001 to about 100; from about 0.005 to about 50; from about 0.001 to about 10; from about 0.01 to about 5; from about 0.1 to about 3; or is about 1.9.

[0256] In another embodiment, the invention is directed to a composition comprising a human IgG2 antibody, a pharmaceutically acceptable chelating agent, and histidine; wherein the molar concentration of the antibody ranges from about 0.0006 millimolar to about 1.35 millimolar, the molar concentration of the chelating agent ranges from about 0.003 millimolar to about 50 millimolar, and the molar concentration of histidine ranges from about 5 millimolar to about 20 millimolar; and wherein the molar ratio of antibody to chelating agent ranges from about 0.005 to about 50; from about 0.001 to about 10; from about 0.01 to about 5; from about 0.1 to about 3; or is about 1.9.

[0257] In another embodiment, the invention is directed to a composition comprising a human IgG2 antibody, a pharmaceutically acceptable chelating agent, and histidine; wherein the molar concentration of the antibody ranges from about 0.0006 millimolar to about 1.35 millimolar, the molar concentration of the chelating agent ranges from about 0.003 millimolar to about 50 millimolar, and the molar concentration of histidine ranges from about 5 millimolar to about 20 millimolar; and wherein the molar ratio of antibody to chelating agent ranges from about 0.001 to about 10; from about 0.01 to about 5; from about 0.1 to about 3; or is about 1.9.

[0258] In another embodiment, the invention is directed to a composition comprising a human IgG2 antibody, a pharmaceutically acceptable chelating agent, and histidine; wherein the molar concentration of the antibody ranges from about 0.0006 millimolar to about 1.35 millimolar, the molar concentration of the chelating agent ranges from about 0.003 millimolar to about 50 millimolar, and the molar concentration of histidine is about 20 millimolar; and wherein the molar ratio of antibody to chelating agent ranges from about 0.001 to about 10; from about 0.01 to about 5; from about 0.1 to about 3; or is about 1.9.

[0259] In another embodiment, the invention is directed to a liquid pharmaceutical composition comprising at least one human IgG2 monoclonal antibody and a pharmaceutically acceptable chelating agent, wherein the molar concentration of the antibodies range from about 0.0006 millimolar to about 1.35 millimolar and the molar concentration of the chelating agent ranges from about 0.003 millimolar to about 50 millimolar, and wherein the molar ratio of antibodies to chelating agent ranges from about 0.00001 to about 2000; from about 0.01 to about 500; from about 0.05 to about 100; from about 0.1 to about 10; or is from about 0.5 to about 4.

Methods of Producing Human IgG2 Antibodies and Antibody Producing Cell Lines:

[0260] Antibodies in accordance with the invention can be prepared through the utilization of a transgenic mouse that has a substantial portion of the human antibody producing genome inserted, but that is rendered deficient in the production of endogenous, murine, antibodies. Such mice, then, are capable of producing human immunoglobulin molecules and antibodies and are deficient in the production of murine immunoglobulin molecules and antibodies. Technologies utilized for achieving the same are discussed below.

[0261] It is possible to produce transgenic animals (e.g., mice) that are capable, upon immunization, of producing a full repertoire of human antibodies in the absence of endogenous immunoglobulin production. In particular, however, several embodiments of the transgenic production of mice and antibodies therefrom are disclosed in U.S. Pat. No. 6,682,736 to Hanson, et al., U.S. Published Application No. 20050059113 to Bedian, et al. and International Patent Application Number PCT/US2005/000370. Through the use of such technology, fully human antibodies that bind to human antigens, such as M-CSF, CTLA-4, and MAdCAM, and hybridomas producing such antibodies, can be prepared by one of skill in the art.

[0262] Human antibodies avoid potential problems associated with antibodies that possess murine or rat variable and/or constant regions. The presence of such murine or rat derived proteins can lead to the rapid clearance of the antibodies or can lead to the generation of an immune response against the antibody by a subject that receives administration of such antibodies.

[0263] For example, it has been described that the homozygous deletion of the antibody heavy-chain joining region (J_H) gene in chimeric and germ-line mutant mice results in complete inhibition of endogenous antibody production. Transfer of the human germ-line immunoglobulin gene array in such germ-line mutant mice will result in the production of human antibodies upon antigen challenge. See, e.g., Jakobovits et al, *Proc. Natl. Acad. Sci. USA,* 90:2551 (1993); Jakobovits et al., *Nature,* 362:255-258 (1993); Bruggermann et al., *Year in Immuno.,* 7:33 (1993); and Duchosal et al., *Nature* 355:258 (1992). Human antibodies can also be derived from phage-display libraries (Hoogenboom et al., *J. Mol. Biol.,* 227:381 (1991); Marks et al., *J. Mol. Biol.,* 222:581-597 (1991); Vaughan et al., *Nature Biotech* 14:309 (1996)).

[0264] In some embodiments, the human IgG2 antibodies can be produced by immunizing a non-human transgenic animal, e.g., XENOMOUSE™ mice, whose genome comprises human immunoglobulin genes so that the recombinant mouse produces human antibodies. XENOMOUSE™ mice are engineered mouse strains that comprise large fragments of human immunoglobulin heavy chain and light chain loci and are deficient in mouse antibody production. XENOMOUSE™ mice produce an adult-like human repertoire of fully human antibodies and generate antigen-specific human antibodies. In some embodiments, the XENOMOUSE™ mice contain approximately 80% of the human antibody V gene repertoire through introduction of megabase sized, germline configuration yeast artificial chromosome (YAC) fragments of the human heavy chain loci and kappa light chain loci. In other embodiments, XENOMOUSE™ mice further contain approximately all of the lambda light chain locus. See, e.g., Green et al., *Nature Genetics* 7:13-21 (1994) and U.S. Pat. Nos. 5,916,771, 5,939,598, 5,985,615, 5,998,209, 6,075,181, 6,091,001, 6,114,598, 6,130,364, 6,162,963 and 6,150,584. See also WO 91/10741, WO 94/02602, WO 96/34096, WO 96/33735, WO 98/16654, WO 98/24893, WO 98/50433, WO 99/45031, WO 99/53049, WO 00/09560, and WO 00/037504.

[0265] In some embodiments, the non-human animal comprising human immunoglobulin genes are animals that have a human immunoglobulin "minilocus". In the minilocus approach, an exogenous Ig locus is mimicked through the inclusion of individual genes from the Ig locus. Thus, one or more V_H genes, one or more DH genes, one or more J_H genes,

a mu constant domain, and a second constant domain (preferably a gamma constant domain) are formed into a construct for insertion into an animal. This approach is described, inter alla, in U.S. Pat. Nos. 5,545,807, 5,545,806, 5,569,825, 5,625,126, 5,633,425, 5,661,016, 5,770,429, 5,789,650, 5,814,318, 5,591,669, 5,612,205, 5,721,367, 5,789,215, and 5,643,763.

[0266] Therefore, in some embodiments, human antibodies can be produced by immunizing a non-human animal comprising in its genome some or all of human immunoglobulin heavy chain and light chain loci with a desired antigen.

[0267] In some embodiments, the desired antigen is isolated and/or purified. In a preferred embodiment, the antigen is a human antigen. In some embodiments, the antigen is a fragment of the desired antigen. In some embodiments, the fragment comprises at least one epitope of the desired antigen. In other embodiments, the antigen is a cell that expresses or overexpresses the antigen or an immunogenic fragment thereof on its surface. In still other embodiments, the antigen is an antigen fusion protein. The desired antigen can be purified from natural sources using known techniques. In addition, many recombinant antigens are commercially available.

[0268] In a preferred embodiment, the non-human animal is a XENOMOUSE™ animal (Abgenix Inc., Fremont, Calif.). Another non-human animal that may be used is a transgenic mouse produced by Medarex (Medarex, Inc., Princeton, N.J.).

[0269] Immunization of animals can be by any method known in the art. See, e.g., Harlow and Lane, *Antibodies: A Laboratory Manual*, New York: Cold Spring Harbor Press, 1990. Methods for immunizing non-human animals such as mice, rats, sheep, goats, pigs, cattle and horses are well known in the art. See, e.g., Harlow and Lane, supra, and U.S. Pat. No. 5,994,619. In a preferred embodiment, the desired antigen is administered with an adjuvant to stimulate the immune response. Exemplary adjuvants include complete or incomplete Freund's adjuvant, RIBI (muramyl dipeptides) or ISCOM (immunostimulating complexes). Such adjuvants may protect the polypeptide from rapid dispersal by sequestering it in a local deposit, or they may contain substances that stimulate the host to secrete factors that are chemotactic for macrophages and other components of the immune system. Preferably, if a polypeptide is being administered, the immunization schedule can involve two or more administrations of the polypeptide, spread out over several weeks.

[0270] After immunization of an animal with the antigen, the antibodies and/or antibody-producing cells can be obtained from the animal. In some embodiments, the fully human IgG2 antibody-containing serum is obtained from the animal by bleeding or sacrificing the animal. The serum may be used as it is obtained from the animal, an immunoglobulin fraction may be obtained from the serum, or the antibodies may be purified from the serum.

[0271] In some embodiments, antibody-producing immortalized cell lines are prepared from cells isolated from the immunized animal. After immunization, the animal is sacrificed and lymph node and/or splenic B cells are immortalized. Methods of immortalizing cells include, but are not limited to, transfecting them with oncogenes, infecting them with an oncogenic virus, cultivating them under conditions that select for immortalized cells, subjecting them to carcinogenic or mutating compounds, fusing them with an immortalized cell, e.g., a myeloma cell, and inactivating a tumor suppressor gene. See, e.g., Harlow and Lane, supra. In a preferred embodiment, the immunized animal is a non-human animal that expresses human immunoglobulin genes and the splenic B cells are fused to a myeloma cell line from the same species as the non-human animal. In a more preferred embodiment, the immunized animal is a XENOMOUSE™ animal and the myeloma cell line is a non-secretory mouse myeloma. In an even more preferred embodiment, the myeloma cell line is P3-X63-AG8-653. If fusion with myeloma cells is used, the myeloma cells preferably do not secrete immunoglobulin polypeptides (a non-secretory cell line). Immortalized cells are screened using the desired antigen, a portion thereof, or a cell expressing the desired antigen. In a preferred embodiment, the initial screening is performed using an enzyme-linked immunoassay (ELISA) or a radioimmunoassay. An example of ELISA screening is provided in WO 00/37504.

[0272] The fully human IgG2 antibody-producing cells, e.g., hybridomas, are selected, cloned and further screened for desirable characteristics, including robust growth, high antibody production and desirable antibody characteristics, as discussed further below. Hybridomas can be expanded in vivo in syngeneic animals, in animals that lack an immune system, e.g., nude mice, or in cell culture in vitro. Methods of selecting, cloning and expanding hybridomas are well known to those of ordinary skill in the art.

[0273] As will be appreciated, antibodies in accordance with the present invention can be recombinantly expressed in cell lines other than hybridoma cell lines. Nucleic acid sequences encoding the cDNAs or genomic clones for the particular antibodies can be used for transformation of a suitable mammalian or nonmammalian host cells.

[0274] The present invention also encompasses nucleic acid molecules encoding the human IgG2 antibodies. In some embodiments, different nucleic acid molecules encode a heavy chain and a light chain of the human IgG2 immunoglobulin. In other embodiments, the same nucleic acid molecule encodes a heavy chain and a light chain of the human IgG2 immunoglobulin. In one embodiment, the nucleic acid encodes the human IgG2 antibody of the invention.

[0275] A nucleic acid molecule encoding the heavy or entire light chain of the human IgG2 antibody or portions thereof can be isolated from any source that produces such antibody. In various embodiments, the nucleic acid molecules are isolated from a B cell isolated from an animal immunized with the desired antigen or from an immortalized cell derived from such a B cell that expresses the human IgG2 antibody. Methods of isolating mRNA encoding an antibody are well-known in the art. See, e.g., Sambrook, et al., *Molecular Cloning* 3rd Ed. Vol. 3 (1989). The mRNA may be used to produce cDNA for use in the polymerase chain reaction (PCR) or cDNA cloning of antibody genes. In a preferred embodiment, the nucleic acid molecule is isolated from a hybridoma that has as one of its fusion partners a human immunoglobulin-producing cell from a non-human transgenic animal. In an even more preferred embodiment, the human immunoglobulin producing cell is isolated from a XENOMOUSE™ animal. In another embodiment, the human immunoglobulin-producing cell is from a non-human, non-mouse transgenic animal, as described above. In another embodiment, the nucleic acid is isolated from a non-human, non-transgenic animal. The nucleic acid molecules isolated from a non-human animal may be used, e.g., for humanized antibodies.

[0276] In some embodiments, a nucleic acid encoding a heavy chain of a human IgG2 antibody of the invention can comprise a nucleotide sequence encoding a V_H domain of the

invention joined in-frame to a nucleotide sequence encoding a heavy chain constant domain from any source. Similarly, a nucleic acid molecule encoding a light chain of an anti-CTLA-4 antibody of the invention can comprise a nucleotide sequence encoding a V_L domain of the invention joined in-frame to a nucleotide sequence encoding a light chain constant domain from any source.

[0277] In a further aspect of the invention, nucleic acid molecules encoding the variable domain of the heavy (V_H) and light (V_L) chains are "converted" to full-length antibody genes. In one embodiment, nucleic acid molecules encoding the V_H or V_L domains are converted to full-length antibody genes by insertion into an expression vector already encoding heavy chain constant (C_H) or light chain (C_L) constant domains, respectively, such that the V_H segment is operatively linked to the C_H segment(s) within the vector, and the V_L segment is operatively linked to the C_L segment within the vector. In another embodiment, nucleic acid molecules encoding the V_H and/or V_L domains are converted into full-length antibody genes by linking, e.g., ligating, a nucleic acid molecule encoding a V_H and/or V_L domains to a nucleic acid molecule encoding a C_H and/or C_L domain using standard molecular biological techniques. Nucleic acid sequences of human heavy and light chain immunoglobulin constant domain genes are known in the art. See, e.g., Kabat et al., *Sequences of Proteins of Immunological Interest,* 5th Ed., NIH Publ. No. 91-3242, 1991. Nucleic acid molecules encoding the full-length heavy and/or light chains may then be expressed from a cell into which they have been introduced and the human IgG2 antibody isolated.

[0278] The present invention also provides vectors comprising nucleic acid molecules that encode the heavy chain of the human IgG2 antibody of the invention or an antigen-binding portion thereof. The invention also provides vectors comprising nucleic acid molecules that encode the light chain of such antibodies or antigen-binding portion thereof. The invention further provides vectors comprising nucleic acid molecules encoding fusion proteins, modified antibodies, antibody fragments, and probes thereof.

[0279] In some embodiments, the human IgG2 antibodies, or antigen-binding portions of the invention are expressed by inserting DNAs encoding partial or full-length light and heavy chains, obtained as described above, into expression vectors such that the genes are operatively linked to necessary expression control sequences such as transcriptional and translational control sequences. Expression vectors include plasmids, retroviruses, adenoviruses, adeno-associated viruses (AAV), plant viruses such as cauliflower mosaic virus, tobacco mosaic virus, cosmids, YACs, EBV derived episomes, and the like. The antibody gene is ligated into a vector such that transcriptional and translational control sequences within the vector serve their intended function of regulating the transcription and translation of the antibody gene. The expression vector and expression control sequences are chosen to be compatible with the expression host cell used. The antibody light chain gene and the antibody heavy chain gene can be inserted into separate vectors. In a preferred embodiment, both genes are inserted into the same expression vector. The antibody genes are inserted into the expression vector by standard methods (e.g., ligation of complementary restriction sites on the antibody gene fragment and vector, or blunt end ligation if no restriction sites are present).

[0280] A convenient vector is one that encodes a functionally complete human C_H or C_L immunoglobulin sequence, with appropriate restriction sites engineered so that any V_H or V_L sequence can easily be inserted and expressed, as described above. In such vectors, splicing usually occurs between the splice donor site in the inserted J region and the splice acceptor site preceding the human C domain, and also at the splice regions that occur within the human C_H exons. Polyadenylation and transcription termination occur at native chromosomal sites downstream of the coding regions. The recombinant expression vector also can encode a signal peptide that facilitates secretion of the antibody chain from a host cell. The antibody chain gene may be cloned into the vector such that the signal peptide is linked in-frame to the amino terminus of the immunoglobulin chain. The signal peptide can be an immunoglobulin signal peptide or a heterologous signal peptide (i.e., a signal peptide from a non-immunoglobulin protein).

[0281] In addition to the antibody chain genes, the recombinant expression vectors of the invention carry regulatory sequences that control the expression of the antibody chain genes in a host cell. It will be appreciated by those skilled in the art that the design of the expression vector, including the selection of regulatory sequences may depend on such factors as the choice of the host cell to be transformed, the level of expression of protein desired, etc. Preferred regulatory sequences for mammalian host cell expression include viral elements that direct high levels of protein expression in mammalian cells, such as promoters and/or enhancers derived from retroviruses (such as retroviral LTRs), cytomegalovirus (CMV) (such as the CMV promoter/enhancer), Simian Virus 40 (SV40) (such as the SV40 promoter/enhancer), adenovirus, (e.g., the adenovirus major late promoter (AdMLP)), polyoma and strong mammalian promoters such as native immunoglobulin and actin promoters. For further description of viral regulatory elements, and sequences thereof, see e.g., U.S. Pat. No. 5,168,062, U.S. Pat. No. 4,510,245 and U.S. Pat. No. 4,968,615. Methods for expressing antibodies in plants, including a description of promoters and vectors, as well as transformation of plants is known in the art. See, e.g., U.S. Pat. No. 6,517,529, herein incorporated by reference. Methods of expressing polypeptides in bacterial cells or fungal cells, e.g., yeast cells, are also well known in the art.

[0282] In addition to the antibody chain genes and regulatory sequences, the recombinant expression vectors of the invention may carry additional sequences, such as sequences that regulate replication of the vector in host cells (e.g., origins of replication) and selectable marker genes. The selectable marker gene facilitates selection of host cells into which the vector has been introduced (see e.g., U.S. Pat. Nos. 4,399,216, 4,634,665 and 5,179,017). For example, typically the selectable marker gene confers resistance to drugs, such as G418, hygromycin or methotrexate, on a host cell into which the vector has been introduced. Preferred selectable marker genes include the dihydrofolate reductase (DHFR) gene (for use in DHFR-host cells with methotrexate selection/amplification), the neomycin resistance gene (for G418 selection), and the glutamine synthetase gene.

[0283] Nucleic acid molecules encoding the fully human IgG2 antibodies and vectors comprising these nucleic acid molecules can be used for transformation of a suitable mammalian, plant, bacterial or yeast host cell. Antibodies of the invention can be produced transgenically through the generation of a mammal or plant that is transgenic for the immuno-

globulin heavy and light chain sequences of interest and production of the antibody in a recoverable form therefrom.

[0284] Transformation can be by any known method for introducing polynucleotides into a host cell, including, for example packaging the polynucleotide in a virus (or into a viral vector) and transducing a host cell with the virus (or vector) or by transfection procedures known in the art, as exemplified by U.S. Pat. Nos. 4,399,216, 4,912,040, 4,740,461, and 4,959,455. The transformation procedure used depends upon the host to be transformed. Methods for introduction of heterologous polynucleotides into mammalian cells are well known in the art and include, but are not limited to, dextran-mediated transfection, calcium phosphate precipitation, polybrene mediated transfection, protoplast fusion, electroporation, particle bombardment, encapsulation of the polynucleotide(s) in liposomes, peptide conjugates, dendrimers, and direct microinjection of the DNA into nuclei.

[0285] Mammalian cell lines available as hosts for expression are well known in the art and include many immortalized cell lines available from the American Type Culture Collection (ATCC), including but not limited to Chinese hamster ovary (CHO) cells, NSO cells, HeLa cells, baby hamster kidney (BHK) cells, monkey kidney cells (COS), human hepatocellular carcinoma cells (e.g., Hep G2), and a number of other cell lines. Non-mammalian cells including but not limited to bacterial, yeast, insect, and plants can also be used to express recombinant antibodies. Site directed mutagenesis of the antibody CH2 domain to eliminate glycosylation may be preferred in order to prevent changes in either the immunogenicity, pharmacokinetic, and/or effector functions resulting from non-human glycosylation. The expression methods are selected by determining which system generates the highest expression levels and produce antibodies with the desired antigen-binding properties.

[0286] Further, expression of antibodies of the invention (or other moieties therefrom) from production cell lines can be enhanced using a number of known techniques. For example, the glutamine sythetase and DHFR gene expression systems are common approaches for enhancing expression under certain conditions. High expressing cell clones can be identified using conventional techniques, such as limited dilution cloning and Microdrop technology. The Glutamine Synthetase system is discussed in whole or part in connection with European Patent Nos. 0 216 846, 0 256 055, and 0 323 997 and European Patent Application No. 89303964.4.

[0287] In connection with the transgenic production in mammals, antibodies can also be produced in, and recovered from, the milk of goats, cows, or other mammals. See, e.g., U.S. Pat. Nos. 5,827,690, 5,756,687, 5,750,172, and 5,741,957.

[0288] The human IgG2 antibodies expressed in cell lines as described above may be purified and/or isolated from the associated cellular material. The antibodies may be present in whole cells, in a cell lysate, or in a partially purified or substantially pure form. Purification is performed in order to eliminate other cellular components or other contaminants, e.g. other cellular nucleic acids or proteins, by standard techniques, including alkaline/SDS treatment, column chromatography and others well known in the art. See Ausubel, F., et al., ed. Current Protocols in Molecular Biology, Greene Publishing and Wiley Interscience, New York (1987).

[0289] In the present invention, it is possible that the human IgG2 antibodies of the present invention expressed by different cell lines or in transgenic animals will have different glycosylation patterns from each other. However, all of the human IgG2 antibodies encoded by the nucleic acids and amino acids provided herein are considered part of the instant invention, regardless of their glycosylation pattern or modification or deletion thereof. Thus, for purposes of the present invention, the human IgG2 antibodies may be glycosylated or non-glycosylated. When the human IgG2 antibodies are glycosylated they may have any possible glycosylation pattern. Moreover, each heavy chain within one antibody may have the same glycosylation pattern or the two heavy chains may have differing glycosylation patterns. Site directed mutagenesis of the antibody CH2 domain to eliminate glycosylation is also encompassed by the present invention in order to prevent changes in either the immunogenicity, pharmacokinetic, and/or effector functions resulting from non-human glycosylation.

[0290] As used herein, the term "glycosylation" means the pattern of carbohydrate units that are covalently attached to an antibody. When it is said that the human IgG2 antibodies herein have a particular glycosylation pattern, it is meant that the majority of the referenced human IgG2 antibodies have that particular glycosylation pattern. In other aspects, when it is said that the human IgG2 antibodies herein have a particular glycosylation pattern, it is meant that greater than or equal to 50%, 75%, 90%, 95%, 99% or 100% of the referenced human IgG2 antibodies have that particular glycosylation pattern.

[0291] The human IgG2 antibodies of the present invention also encompass glycosylation variants thereof (e.g., by insertion of a glycosylation site or deletion of any glycosylation site by deletion, insertion or substitution of suitable amino acid residues).

[0292] Glycosylation of polypeptides is typically either N-linked or O-linked. Glycosylation of antibody polypeptides is typically N-linked and forms a biantennary structure. N-linked refers to the attachment of the carbohydrate moiety to the side chain of an asparagine residue. The tri-peptide sequences asparagine-X-serine and asparagine-X-threonine, where X is any amino acid except proline, are the recognition sequences for enzymatic attachment of the carbohydrate moiety to the asparagine side chain. Thus, the presence of either of these tri-peptide sequences in an antibody creates a potential glycosylation site.

[0293] The three distinct structures of biantennary glycans are designated "G0", "G1" and "G2" having zero, one, or two, respectively, terminal galactose residues on the nonreducing end of the glycan. See Jefferis et al., *Biochem. J.,* 268, 529-537 (1990). In some cases, the glycan structure may also have a fucose residue linked to an N-acetylglucosamine, which is covalently bonded to the asparagine amino acid (e.g., position 297) found in the antibody. When the fucose (F) is present, the biantennary glycan nomenclature is changed to "G0F", "G1F", or "G2F" depending upon the number of terminal galactose residues. See Teillaud, *Expert Opin. Biol. Ther.,* 5(Suppl. 1):S15-S27 (2005). Furthermore, when the antibody contains both of the two heavy chains, the glycan nomenclature is repeated for each of the two heavy chains. Thus, the "G0F,G0F" glycoform is a species in which both heavy chains have the G0 glycan attached and each G0 glycan has a fucose (F) residue linked to an N-acetylglucosamine. The "G0F,G1F" glycoform is a species in which one of the heavy chains has the G0 glycan attached and the other heavy chain has the G1 glycan attached with each G0 glycan and G1 glycan having a fucose (F) residue linked to an N-acetylglu-

cosamine. In certain embodiments, the human IgG2 antibodies have a glycosylation pattern that is selected from the group consisting of "G0F,G0F"; "G0F,G1F"; "G1F,G1F"; "G1F, G2F"; and mixtures thereof.

Routes of Administration and Dosages:

[0294] The compositions of this invention may be in liquid solutions (e.g., injectable and infusible solutions). The preferred form depends on the intended mode of administration and therapeutic application. Typical preferred compositions are in the form of injectable or infusible solutions, such as compositions similar to those used for passive immunization of humans. The preferred mode of administration is parenteral (e.g., intravenous, subcutaneous, intraperitoneal, intramuscular, intradermal, and intrasternally) or by infusion techniques, in the form of sterile injectable liquid or olagenous suspensions. As will be appreciated by the skilled artisan, the route and/or mode of administration will vary depending upon the desired results. In a preferred embodiment, the antibody is administered by intravenous infusion or injection. In another preferred embodiment, the antibody is administered by intramuscular or subcutaneous injection. Therapeutic compositions typically are sterile and stable under the conditions of manufacture and storage.

[0295] The composition can be formulated as a solution, microemulsion, dispersion, or liposome. Sterile injectable solutions can be prepared by incorporating the human IgG2 antibody in the required amount in an appropriate diluent with one or a combination of ingredients enumerated above, as required, followed by sterilization (e.g., filter sterilization). Generally, dispersions are prepared by incorporating the active compound into a sterile vehicle that contains a basic dispersion medium and the required other ingredients from those enumerated above. Such suspensions may be formulated according to the known art using those suitable dispersing of wetting agents and suspending agents or other acceptable agents. The sterile injectable preparation may also be a sterile injectable solution or suspension in a non-toxic parenterally acceptable diluent or solvent, for example as a solution in 1,3-butanediol. Among the acceptable vehicles and solvents that may be employed are water, Ringer's solution and isotonic sodium chloride solution. In addition, sterile, fixed oils are conventionally employed as a solvent or suspending medium. For this purpose, any bland fixed oil may be employed, including synthetic mono- or diglycerides. In addition, n−3 polyunsaturated fatty acids may find use in the preparation of injectables.

[0296] In the case of sterile powders for the preparation of sterile injectable solutions, the preferred methods of preparation are vacuum drying and freeze-drying that yields a powder of the active ingredient plus any additional desired ingredient from a previously sterile-filtered solution thereof. The proper fluidity of a solution can be maintained, for example, by the use of a coating such as lecithin, by the maintenance of the required particle size in the case of dispersion and by the use of surfactants.

[0297] Prolonged absorption of injectable compositions can be brought about by including in the composition an agent that delays absorption, for example, monostearate salts and gelatin or by formulating the composition into prolonged absorption forms such as, depots, liposomes, polymeric microspheres, polymeric gels, and implants.

[0298] Other methods for administration of the antibodies described herein include dermal patches that release the medications directly into a subject's skin. Such patches can contain the antibodies of the present invention in an optionally buffered, liquid solution, dissolved and/or dispersed in an adhesive, or dispersed in a polymer.

[0299] Still other methods for administration of the antibodies described herein include liquid opthalmological drops for the eyes.

[0300] The antibody may be administered once, but more preferably is administered multiple times. For example, the antibody may be administered from once daily to once every six months or longer. The administering may be on a schedule such as three times daily, twice daily, once daily, once every two days, once every three days, once weekly, once every two weeks, once every month, once every two months, once every three months and once every six months.

[0301] The antibody may also be administered continuously via a minipump. The antibody may be administered at the site of the diseased body part or at a site distant from the site of the diseased body part. The antibody may be administered once, at least twice or for at least the period of time until the disease is treated, palliated or cured. The antibody generally may be administered for as long as the disease is present. The antibody typically would be administered as part of a pharmaceutical composition as described supra.

[0302] The compositions of the invention may include a therapeutically effective amount or a prophylactically effective amount of an antibody or antigen-binding portion of the invention. In preparing the composition, the therapeutically effective amount of the human IgG2 antibody present in the composition can be determined, for example, by taking into account the desired dose volumes and mode(s) of administration, the nature and severity of the condition to be treated, and the age and size of the subject.

[0303] Exemplary, non-limiting dose ranges for administration of the pharmaceutical compositions of the present invention to a subject are from about 0.01 mg/kg to about 200 mg/kg (expressed in terms of milligrams (mg) of human IgG2 antibody administered per kilogram (kg) of subject weight), from about 0.1 mg/kg to about 100 mg/kg, from about 1.0 mg/kg to about 50 mg/kg, from about 5.0 mg/kg to about 20 mg/kg, or about 15 mg/kg. For purposes of the present invention, an average human subject weighs about 70 kg. Ranges intermediate to any of the dosages cited herein, e.g., about 0.02 mg/kg-199 mg/kg, are also intended to be part of this invention. For example, ranges of values using a combination of any of the recited values as upper and/or lower limits are intended to be included.

[0304] Dosage regimens can also be adjusted to provide the optimum desired response (e.g., a therapeutic or prophylactic response) by administering several divided doses to a subject over time or the dose can be proportionally reduced or increased as indicated by the exigencies of the therapeutic situation. It is especially advantageous to formulate parenteral compositions in dosage unit form for ease of administration and uniformity of dosage.

[0305] Dosage unit form as used herein refers to physically discrete units suited as unitary dosages for the mammalian subjects to be treated; each unit containing a predetermined quantity of active compound calculated to produce the desired therapeutic effect in association with the required pharmaceutical carrier. The specification for the dosage unit forms of the invention are dictated by and directly dependent on (a) the unique characteristics of the human IgG2 antibody or portion and the particular therapeutic or prophylactic effect

to be achieved, and (b) the limitations inherent in the art of compounding such an antibody for the treatment of sensitivity in individuals.

[0306] The liquid compositions of the present invention can be prepared as unit dosage forms. For example, a unit dosage per vial may contain from 1 to 1000 milliliters (mls) of different concentrations of a human IgG2 antibody. In other embodiments, a unit dosage per vial may contain about 1 ml, 2 ml, 3 ml, 4 ml, 5 ml, 6 ml, 7 ml, 8 ml, 9 ml, 10 ml, 15 ml, 20 ml, 30 ml; 40 ml, 50 ml or 100 ml of different concentrations of a human IgG2 antibody. If necessary, these preparations can be adjusted to a desired concentration by adding a sterile diluent to each vial. The liquid compositions of the present invention can also be prepared as unit dosage forms in sterile bags or containers, which are suitable for connection to an intravenous administration line or catheter.

Stability Assessment:

[0307] The present invention comprises stable liquid pharmaceutical compositions comprising a human IgG2 antibody as described herein and a pharmaceutically acceptable chelating agent. A stable composition is desirable to maintain or resist changes in, for example, product appearance and integrity (including physical or chemical degradation potentially leading to a reduction in biological activity). Various analytical techniques and indicators for measuring protein stability are reported in the literature and a number of these techniques and indicators are reviewed in *Peptide and Protein Drug Delivery,* 247-301, Vincent Lee Ed., Marcel Dekker, Inc., New York, N.Y., Pubs. (1991) and Jones, A. Adv. *Drug Delivery Rev.* 10: 29-90 (1993). In general, the liquid pharmaceutical compositions of the present invention exhibit improved stability when subjected to low storage temperatures over a period of time, and/or when subjected to one or more freeze/thaw cycles.

[0308] In one embodiment, the composition when stored at a temperature from about 2° C. to about 8° C. for at least about 12 months, preferably at least about 18 months and more preferably at least about 24 months, is more stable than an otherwise identical composition lacking the chelating agent that is stored under the same conditions for the same time.

[0309] In another embodiment, the composition when stored at a temperature from about 25° C. to about 30° C. for at least about 3 months, preferably at least 6 months, and more preferably at least about 12 months, is more stable than an otherwise identical composition lacking the chelating agent that is stored under the same conditions for the same time.

[0310] In another embodiment, the composition when stored at a temperature of about 40° C. for at least about 1 month, preferably at least about 2 months, more preferably at least about 3 months, and yet more preferably at least about 26 weeks, is more stable than an otherwise identical composition lacking the chelating agent that is stored under the same conditions for the same time.

[0311] In another embodiment, the composition when stored at a temperature from about 2° C. to about 8° C. for at least about 12 months, preferably at least about 18 months and more preferably at least about 24 months, is more stable than an isotonic composition lacking the chelating agent that is stored under the same conditions for the same time.

[0312] In another embodiment, the composition when stored at a temperature from about 25° C. to about 30° C. for at least about 3 months, preferably at least 6 months, and more preferably at least about 12 months, is more stable than an isotonic composition lacking the chelating agent that is stored under the same conditions for the same time.

[0313] In another embodiment, the composition when stored at a temperature of about 40° C. for at least about 1 month, preferably at least about 2 months, more preferably at least about 3 months, and yet more preferably at least about 26 weeks, is more stable than an isotonic composition lacking the chelating agent that is stored under the same conditions for the same time.

[0314] As used herein, the term "a freeze/thaw cycle" refers to techniques for using a liquid antibody sample after frozen storage, wherein the temperature of the sample is lowered to a temperature of 0° C. or lower in order to freeze the liquid sample, and then subjecting the sample to a temperature which will restore its liquid state for a sufficient period of time to permit use of the sample, followed by and return to frozen storage, preferably at a temperature of 0° C. or lower. As used herein, the term "frozen storage" refers to freezing and maintaining a previously liquid antibody sample at a temperature of 0° C. or below, and preferably −20° C. or lower.

[0315] In one embodiment, the composition when subjected to at least 1 freeze/thaw cycle, preferably at least 2 freeze/thaw cycles, more preferably at least 3 freeze/thaw cycles, still more preferably at least 4 freeze/thaw cycles, still more preferably at least 5 freeze/thaw cycles, and still more preferably at least 6 freeze/thaw cycles, is more stable than an otherwise identical composition lacking the chelating agent that is subjected to the same freeze/thaw conditions.

[0316] In another embodiment, the composition when subjected to at least 1 freeze/thaw cycle, preferably at least 2 freeze/thaw cycles, more preferably at least 3 freeze/thaw cycles, still more preferably at least 4 freeze/thaw cycles, still more preferably at least 5 freeze/thaw cycles, and still more preferably at least 6 freeze/thaw cycles, is more stable than an isotonic composition lacking the chelating agent that is subjected to the same freeze/thaw conditions.

[0317] In another embodiment, the composition satisfies two or more of the following conditions:

[0318] (a) the composition when stored at a temperature from about 2° C. to about 8° C. for at least about 12 months, preferably at least about 18 months and more preferably at least about 24 months, is more stable than an isotonic composition lacking the chelating agent that is stored under the same conditions for the same time;

[0319] (b) the composition when stored at a temperature from about 25° C. to about 30° C. for at least about 3 months, preferably at least 6 months, and more preferably at least about 12 months, is more stable than an isotonic composition lacking the chelating agent that is stored under the same conditions for the same time;

[0320] (c) the composition when stored at a temperature of about 40° C. for at least about 1 month, preferably at least about 2 months, more preferably at least about 3 months, and yet more preferably at least about 26 weeks, is more stable than an isotonic composition lacking the chelating agent that is stored under the same conditions for the same time; or

[0321] (d) the composition when subjected to at least 1 freeze/thaw cycle, preferably at least 2 freeze/thaw cycles, more preferably at least 3 freeze/thaw cycles, still more preferably at least 4 freeze/thaw cycles, still more preferably at least 5 freeze/thaw cycles, and still more preferably at least 6 freeze/thaw cycles, is more stable than an isotonic composition lacking the chelating agent that is subjected to the same freeze/thaw conditions.

[0322] In another embodiment, the composition satisfies two or more of the following conditions:

[0323] (a) the composition when stored at a temperature from about 2° C. to about 8° C. for at least about 12 months, preferably at least about 18 months and more preferably at least about 24 months, is more stable than an otherwise identical composition lacking the chelating agent that is stored under the same conditions for the same time;

[0324] (b) the composition when stored at a temperature from about 25° C. to about 30° C. for at least about 3 months, preferably at least 6 months, and more preferably at least about 12 months, is more stable than an otherwise identical composition lacking the chelating agent that is stored under the same conditions for the same time;

[0325] (c) the composition when stored at a temperature of about 40° C. for at least about 1 month, preferably at least about 2 months, more preferably at least about 3 months, and yet more preferably at least about 26 weeks, is more stable than an otherwise identical composition lacking the chelating agent that is stored under the same conditions for the same time; or

[0326] (d) the composition when subjected to at least 1 freeze/thaw cycle, preferably at least 2 freeze/thaw cycles, more preferably at least 3 freeze/thaw cycles, still more preferably at least 4 freeze/thaw cycles, still more preferably at least 5 freeze/thaw cycles, and still more preferably at least 6 freeze/thaw cycles, is more stable than an otherwise identical composition lacking the chelating agent that is subjected to the same freeze/thaw conditions.

[0327] In another embodiment, the composition satisfies three or more of the conditions discussed immediately above.

[0328] For purposes of the present application, antibody aggregation, antibody fragmentation, and/or composition discoloration, for example, can be used as indicators of the stability of the composition. In general, the compositions of the present invention exhibit a lower level of at least one of antibody aggregation, antibody fragmentation and composition discoloration when subjected to one or more of the above-described storage or freeze/thaw conditions relative to isotonic compositions lacking the chelating agent that are subjected to the same conditions. In other embodiments, the compositions of the present invention exhibit a lower level of at least one of antibody aggregation, antibody fragmentation and composition discoloration when subjected to one or more of the above-described storage or freeze/thaw conditions relative to otherwise identical compositions lacking the chelating agent that are subjected to the same conditions.

[0329] Protein aggregation in a composition, such as a liquid pharmaceutical composition, can be measured by various methods known in the art. Such methods include gel filtration chromatography to separate proteins on the basis of their molecular weight. A "gel" is a matrix of water and a polymer, such as agarose or polymerized acrylamide. The present invention also encompasses the use of gel filtration HPLC (high performance liquid chromatography). Other recognized methods of measuring aggregation include cation exchange chromatography, which is the general liquid chromatographic technique of ion-exchange chromatography utilizing anion columns. The cations exchanged in the present invention are from the protein molecules. Since multivalent protein aggregates may have some multiple of the net charge of the single-chain antigen-binding protein, the aggregates can be retained more strongly, and may be separated from the single-chain molecules. A preferred cationic exchanger is a polyaspartic acid column. Thus, a monomeric protein can be readily distinguished from an aggregate. However, those of ordinary skill in the art will realize that aggregation assays of the invention are not limited to any particular type of chromatography column, so long as it is capable of separating the two forms of protein molecules.

[0330] Protein fragmentation in a liquid pharmaceutical composition can be measured by various methods known in the art. Such methods include, for example, size exclusion chromatography, ultraviolet detection (e.g., at 214 nanometers), SDS-PAGE and/or matrix-assisted laser desorption ionization/time-of-flight mass spectrometry (MALDI/TOF MS). Protein fragmentation resulting in a charge alteration (e.g., occurring as a result of deamidation) can be evaluated, for example, by ion-exchange chromatography or isoelectric focusing (IEF).

[0331] Composition discoloration generally can be measured by visual observation of the composition itself. The present liquid pharmaceutical compositions comprising a chelating agent generally reduce composition discoloration (e.g., pink or yellow) relative to otherwise identical compositions that do not contain the chelating agent. For purposes of the present invention, the term "discoloration" refers to both changes in color (e.g., from clear and colorless to pink or yellow) and to changes in clarity (e.g., from clear and colorless to turbid, cloudy and/or having particulates). Composition discoloration generally can be measured using additional techniques such as by ultraviolet detection at 214 nanometers and/or by visual comparison against a standard color scale of the compositions with and without the chelating agent. See PhEur 5.0, 2005 Monograph 2.2.2.

[0332] In one embodiment, antibody aggregation is determined after the composition is subjected to at least one of the following conditions:

[0333] (a) the composition is stored at a temperature from about 2° C. to about 8° C. for at least about 12 months, preferably at least about 18 months and more preferably at least about 24 months;

[0334] (b) the composition is stored at a temperature from about 25° C. to about 30° C. for at least about 3 months, preferably at least 6 months, and more preferably at least about 12 months;

[0335] (c) the composition is stored at a temperature of about 40° C. for at least about 1 month, preferably at least about 2 months, more preferably at least about 3 months, and yet more preferably at least about 26 weeks; or

[0336] (d) the composition is subjected to at least 1 freeze/thaw cycle, preferably at least 2 freeze/thaw cycles, more preferably at least 3 freeze/thaw cycles, still more preferably at least 4 freeze/thaw cycles, still more preferably at least 5 freeze/thaw cycles, and still more preferably at least 6 freeze/thaw cycles. Antibody aggregates are then chromatographically separated from the composition (e.g., using HPLC) and the extent of aggregation determined from the resulting chromatogram.

[0337] In one embodiment, the present invention, also provides a composition comprising at least one human IgG2 antibody; and a chelating agent, wherein the antibody is stable at a temperature of about 5° C. for at least about 26 weeks.

[0338] In another embodiment, the present invention also provides a composition comprising at least one human IgG2

antibody; and a chelating agent, wherein the antibody is stable at a temperature of about 25° C. for at least about 26 weeks.

[0339] In another embodiment, the present invention also provides a composition comprising at least one human IgG2 antibody; and a chelating agent, wherein the antibody is stable at a temperature of about 40° C. for at least about 26 weeks.

[0340] The stable compositions of the present invention typically have an aggregate peak area on the chromatogram that is less than or equal to any of the following: about 8.0%, about 7.5%, about 7.0%, about 6.5%, about 6.0%, about 5.5%, about 5.0%, about 4.5%, about 4%, about 3.5%, about 3%, about 2.5%, about 2%, about 1.5%, about 1.0%, about 0.9%, or about 0.8% of the total peak area on the chromatogram.

[0341] In one specific example of this technique for measuring aggregation, the composition is stored for 26 weeks at 40° C. and chromatographic separation is then conducted using SE-HPLC with ultraviolet detection at 214 nanometers. In one specific example of this technique for measuring aggregation, the composition is stored for 24 weeks at 40° C. and chromatographic separation is then conducted using SE-HPLC with ultraviolet detection at 214 nanometers. This technique was used to measure antibody aggregation in Example 11-B where, for example, Composition No. 37-B (containing a chelating agent) exhibited an aggregate peak area on the chromatogram of about 1.1% while Composition 26-B (lacking a chelating agent) exhibited an aggregate peak area on the chromatogram of about 6.4%.

[0342] In general, the difference between the aggregate chromatogram peak area for a stable composition of the present invention and the aggregate chromatogram peak area for an isotonic composition lacking the chelating agent that is subjected to the same conditions is at least about 8.0% or greater, is least about 7.5% or greater, is least about 7.0% or greater, is at least about 6.5% or greater, is at least about 6.0% or greater, is at least about 5.5% or greater, at least about 5.0% or greater, at least about 4.5% or greater, at least about 4% or greater, at least about 3.5% or greater, at least about 3.0% or greater, at least about 2.5% or greater, at least about 2.0% or greater, at least about 1.5% or greater, at least about 1.0% or greater, at least about 0.5% or greater, at least about 0.3% or greater, at least about 0.2% or greater, or at least about 0.1% or greater.

[0343] In another embodiment, the difference between the aggregate chromatogram peak area for a stable composition of the present invention and the aggregate chromatogram peak area for an otherwise identical composition lacking the chelating agent that is subjected to the same conditions is at least about 8.0% or greater, is least about 7.5% or greater, is least about 7.0% or greater, is at least about 6.5% or greater, is at least about 6.0% or greater, is at least about 5.5% or greater, at least about 5.0% or greater, at least about 4.5% or greater, at least about 4% or greater, at least about 3.5% or greater, at least about 3.0% or greater, at least about 2.5% or greater, at least about 2.0% or greater, at least about 1.5% or greater, at least about 1.0% or greater, at least about 0.5% or greater, at least about 0.3% or greater, at least about 0.2% or greater, or at least about 0.1% or greater.

[0344] For example, this difference between Composition 37-B (aggregate peak area on the chromatogram of about 1.1%) and Composition 26-B (aggregate peak area on the chromatogram of about 6.4%) tested in Example 11-C as discussed above is about 5.3%.

[0345] In another embodiment, antibody fragmentation is determined after the composition is subjected to at least one of the following conditions:

[0346] (a) the composition is stored at a temperature from about 2° C. to about 8° C. for at least about 12 months, preferably at least about 18 months and more preferably at least about 24 months;

[0347] (b) the composition is stored at a temperature from about 25° C. to about 30° C. for at least about 3 months, preferably at least 6 months, and more preferably at least about 12 months;

[0348] (c) the composition is stored at a temperature of about 40° C. for at least about 1 month, preferably at least about 2 months, and more preferably at least about 3 months; or

[0349] (d) the composition is subjected to at least 1 freeze/thaw cycle, preferably at least 2 freeze/thaw cycles, more preferably at least 3 freeze/thaw cycles, still more preferably at least 4 freeze/thaw cycles, still more preferably at least 5 freeze/thaw cycles, and still more preferably at least 6 freeze/thaw cycles. Antibody fragments are then chromatographically separated from the composition (e.g., using gel filtration) and the extent of fragmentation determined from the resulting chromatogram. The compositions of the present invention typically have a fragment band volume on the chromatogram that is less than about 9%, less than about 8%, less than about 7%, less than about 6%, less than about 5%, or less than about 4.5% of the total band volume on the chromatogram. In one specific example of this technique for measuring fragmentation, the composition is stored for 24 weeks at 40° C. and then chromatographed using reduced SDS-PAGE (rSDS-PAGE) with band volumes determined by scanning with either a Molecular Dynamics Personal Densitometer PDQC-90 or a Bio-Rad GS800 Imaging Densitometer. This technique was used to measure antibody fragmentation in Example 11-B where, for example, Composition No. 37-B (containing a chelating agent) exhibited a fragment band volume on the chromatogram of about 4.5% while Composition 26-B (lacking a chelating agent) exhibited a fragment band volume on the chromatogram of about 10.1%.

[0350] In general, the difference between the fragment band volume for a stable liquid pharmaceutical composition of the present invention and the fragment band volume for an otherwise identical composition lacking the chelating agent that is subjected to the same conditions is at least about 0.1%, at least about 0.5%, at least about 1%, at least about 2%, at least about 3%, at least about 4%, or at least about 5%. For example, this difference between Composition 37-B (fragment band volume on the chromatogram of about 4.5%) and Composition 26-B (fragment band volume on the chromatogram of about 10.1%) tested in Example 11-B as discussed above is about 5.6%.

[0351] In one embodiment, the present invention also provides a stable composition comprising at least one monoclonal human IgG2 antibody and a stabilizing amount of a chelating agent, wherein after the composition is stored for a period of about 26 weeks at a temperature of about 40° C., one or both of the following conditions are satisfied: the decrease between a fragment chromatogram peak area for the stable composition comprising at least one monoclonal human IgG2 antibody and the chelating agent, and a fragment chromatogram peak area for an isotonic composition lacking the

chelating agent, is at least about 1%; or the decrease between a fragment chromatogram peak area for the stable composition comprising at least one monoclonal human IgG2 antibody and the chelating agent, and a fragment chromatogram peak area for an isotonic composition lacking the chelating agent, is at least about 0.5%.

[0352] In another embodiment, the present invention also provides a stable composition comprising at least one monoclonal human IgG2 antibody and a stabilizing amount of a chelating agent, wherein after the composition is stored for a period of about 26 weeks at a temperature of about 40° C., one or both of the following conditions are satisfied: the decrease between a fragment chromatogram peak area for the stable composition comprising at least one monoclonal human IgG2 antibody and the chelating agent, and a fragment chromatogram peak area for an otherwise identical composition lacking the chelating agent, is at least about 1%; or the decrease between a fragment chromatogram peak area for the stable composition comprising at least one monoclonal human IgG2 antibody and the chelating agent, and a fragment chromatogram peak area for an otherwise identical composition lacking the chelating agent, is at least about 0.5%.

[0353] In another embodiment, the present invention also provides a stable composition comprising at least one monoclonal human IgG2 antibody and a stabilizing amount of a chelating agent, wherein after the composition is stored for a period of about 26 weeks at a temperature of about 40° C., one or both of the following conditions are satisfied: the decrease between a fragment chromatogram peak area for the stable composition comprising at least one monoclonal human IgG2 antibody and the chelating agent, and a fragment chromatogram peak area for an otherwise identical composition lacking the chelating agent, is at least about 3.5% or the decrease between a fragment chromatogram peak area for the stable composition comprising at least one monoclonal human IgG2 antibody and the chelating agent, and a fragment chromatogram peak area for an otherwise identical composition lacking the chelating agent, is at least about 1.7%.

[0354] In another embodiment, the present invention also provides a stable composition comprising at least one monoclonal human IgG2 antibody and a stabilizing amount of a chelating agent, wherein after the composition is stored for a period of about 26 weeks at a temperature of about 40° C., one or both of the following conditions are satisfied: the decrease between a fragment chromatogram peak area for the stable composition comprising at least one monoclonal human IgG2 antibody and the chelating agent, and a fragment chromatogram peak area for an isotonic composition lacking the chelating agent, is at least about 3.5%; or the decrease between a fragment chromatogram peak area for the stable composition comprising at least one monoclonal human IgG2 antibody and the chelating agent, and a fragment chromatogram peak area for an isotonic composition lacking the chelating agent, is at least about 1.7%.

[0355] In another embodiment, the present invention also provides a stable composition comprising at least one monoclonal human IgG2 antibody and a chelating agent, wherein after the composition is stored for a period of about 24 weeks at a temperature of about 40° C., a decrease between an aggregate chromatogram peak area for the stable liquid pharmaceutical composition comprising monoclonal human IgG2 antibodies and the chelating agent; and an aggregate chromatogram peak area for an otherwise identical composition lacking the chelating agent that is stored for a period of about 24 weeks at a temperature of about 40° C., is at least about 2%.

[0356] In another embodiment, the present invention also provides a method for stabilizing at least one monoclonal human IgG2 antibody comprising the method of forming a composition comprising the antibodies and a stabilizing amount of a chelating agent, wherein after the composition is stored for a period of about 26 weeks at a temperature of about 40° C., one or both of the following conditions are satisfied: the decrease between an aggregate chromatogram peak area for the stable composition comprising monoclonal human IgG2 antibodies and the chelating agent, and an aggregate chromatogram peak area for an isotonic composition lacking the chelating agent, is at least about 1%; or the decrease between an aggregate chromatogram peak area for the stable composition comprising monoclonal human IgG2 antibodies and the chelating agent, and an aggregate chromatogram peak area for an isotonic composition lacking the chelating agent, is at least about 0.5%.

[0357] In another embodiment, the present invention also provides a method for stabilizing at least one monoclonal human IgG2 antibody comprising the method of forming a composition comprising the antibodies and a stabilizing amount of a chelating agent, wherein after the composition is stored for a period of about 26 weeks at a temperature of about 40° C., one or both of the following conditions are satisfied: the decrease between an aggregate chromatogram peak area for the stable composition comprising monoclonal human IgG2 antibodies and the chelating agent, and an aggregate chromatogram peak area for an otherwise identical composition lacking the chelating agent, is at least about 1%; or the decrease between an aggregate chromatogram peak area for the stable composition comprising monoclonal human IgG2 antibodies and the chelating agent, and an aggregate chromatogram peak area for an otherwise identical composition lacking the chelating agent, is at least about 0.5%.

[0358] In another embodiment, the present invention also provides a method for stabilizing at least one monoclonal human IgG2 antibody comprising the method of forming a composition comprising the antibodies and a stabilizing amount of a chelating agent, wherein after the composition is stored for a period of about 26 weeks at a temperature of about 40° C., one or both of the following conditions are satisfied: the decrease between an aggregate chromatogram peak area for the stable composition comprising monoclonal human IgG2 antibodies and the chelating agent, and an aggregate chromatogram peak area for an isotonic composition lacking the chelating agent, is at least about 11.6%; and/or the decrease between an aggregate chromatogram peak area for the stable composition comprising monoclonal human IgG2 antibodies and the chelating agent, and a aggregate chromatogram peak area for an isotonic composition lacking the chelating agent, is at least about 4.9%.

[0359] In another embodiment, the present invention also provides a method for stabilizing at least one monoclonal human IgG2 antibody comprising the method of forming a composition comprising the antibodies and a stabilizing amount of a chelating agent, wherein after the composition is stored for a period of about 26 weeks at a temperature of about 40° C., one or both of the following conditions are satisfied: the decrease between an aggregate chromatogram peak area for the stable composition comprising monoclonal human IgG2 antibodies and the chelating agent, and an aggregate

chromatogram peak area for an otherwise identical composition lacking the chelating agent, is at least about 11.6%; and/or the decrease between an aggregate chromatogram peak area for the stable composition comprising monoclonal human IgG2 antibodies and the chelating agent, and a aggregate chromatogram peak area for an otherwise identical composition lacking the chelating agent, is at least about 4.9%.

[0360] Accordingly, the present invention provides a method for stabilizing at least one human IgG2 antibody by combining the antibody in a composition with a chelating agent in an amount, which reduces chemical and/or physical instability of the antibody.

Methods of Treatment:

[0361] Any of the types of antibodies described herein may be used therapeutically in a subject.

[0362] In a preferred embodiment, the subject is a human subject. Alternatively, the subject may be a mammal that expresses an antigen/protein that the human IgG2 antibody cross-reacts with. The antibody may be administered to a non-human mammal expressing an antigen with which the antibody cross-reacts (i.e., a primate) for veterinary purposes or as an animal model of human disease. Such animal models may be useful for evaluating the therapeutic efficacy of antibodies of this invention.

[0363] The present invention provides a method for the treatment of any known or later discovered disease or disorder in a subject, and in certain embodiments, an inflammatory or neoplasia disease in a subject, comprising administering to the subject a liquid pharmaceutical composition comprising a human IgG2 antibody; and a chelating agent alone or in combination with other excipients chosen from a buffer, a tonicity agent, an antioxidant, or a surfactant, and mixtures thereof. In further embodiments, the aforementioned subject is one that is in need of the prevention or treatment of a neoplasia disease. In further embodiments, the aforementioned subject is one that is in need of the prevention or treatment of an inflammatory disease.

[0364] In another embodiment, the present invention also provides a method for the treatment of an inflammatory disease in a subject, comprising administering to the subject a therapeutically effective amount of a liquid pharmaceutical composition comprising at least one pharmaceutically acceptable chelating agent and at least one human IgG2 antibody.

[0365] In another embodiment, the present invention also provides a method for the treatment of a neoplasia disease in a subject, comprising administering to the subject a therapeutically effective amount of a liquid pharmaceutical composition comprising at least one pharmaceutically acceptable chelating agent and at least one human IgG2 antibody.

[0366] In another embodiment, the present invention provides a method for the treatment of an inflammatory or neoplasia disease in a subject, comprising administering to the subject a liquid pharmaceutical composition comprising a human IgG2 antibody; and pharmaceutically acceptable excipient comprising a chelating agent alone or in combination with other excipients chosen from a buffer, a tonicity agent, an antioxidant, or a surfactant, and mixtures thereof.

[0367] In further embodiments, the aforementioned subject is one that is in need of the treatment of an inflammatory disease. In other embodiments, the methods and compositions of the present invention encompass the treatment of various inflammatory diseases, of which, the inflammatory disease may be, but is not limited to inflammatory diseases of the gastrointestinal tract including Crohn's disease, ulcerative colitis, diverticula disease, gastritis, liver disease, primary biliary sclerosis, sclerosing cholangitis. Inflammatory diseases also include but are not limited to abdominal disease (including peritonitis, appendicitis, biliary tract disease), acute transverse myelitis, allergic dermatitis (including allergic skin, allergic eczema, skin atopy, atopic eczema, atopic dermatitis, cutaneous inflammation, inflammatory eczema, inflammatory dermatitis, flea skin, military dermatitis, military eczema, house dust mite skin), ankylosing spondylitis (Reiters syndrome), asthma, airway inflammation, atherosclerosis, arteriosclerosis, biliary atresia, bladder inflammation, breast cancer, cardiovascular inflammation (including vasculitis, rheumatoid nail-fold infarcts, leg ulcers, polymyositis, chronic vascular inflammation, pericarditis, chronic obstructive pulmonary disease), chronic pancreatitis, perineural inflammation, colitis (including amoebic colitis, infective colitis, bacterial colitis, Crohn's colitis, ischemic colitis, ulcerative colitis, idiopathic proctocolitis, inflammatory bowel disease, pseudomembranouscolitis), collagen vascular disorders (rheumatoid arthritis, SLE, progressive systemic sclerosis, mixed connective tissue disease, diabetes mellitus), Crohn's disease (regional enteritis, granulomatous ileitis, ileocolitis, digestive system inflammation), demyelinating disease (including myelitis, multiple sclerosis, disseminated sclerosis, acute disseminated encephalomyelitis, perivenous demyelination, vitamin B12 deficiency, Guillain-Barre syndrome, MS-associated retrovirus), dermatomyositis, diverticulitis, exudative diarrheas, gastritis, granulomatous hepatitis, grenulomatous inflammation, cholecystitis, insulin-dependent diabetes mellitus, liver inflammatory diseases (liver fibrosis primary biliary cirrhosis, hepatitis, sclerosing cholangitis), lung inflammation (idiopathic pulmonary fibrosis, eosinophilic granuloma of the lung, pulmonary histiocytosis X, peribronchiolar inflammation, acute bronchitis), lymphogranuloma venereum, malignant melanoma, mouth/tooth disease (including gingivitis, periodontal disease), mucositis, musculoskeletal system inflammation (myositis), nonalcoholic steatohepatitis (nonalcoholic fatty liver disease), ocular & orbital inflammation (including uveitis, optic neuritis, peripheral rheumatoid ulceration, peripheral corneal inflammation), osteoarthritis, osteomyelitis, pharyngeal inflammation, polyarthritis, proctitis, psoriasis, radiation injury, sarcoidosis, sickle cell neuropathy, superfipial thrombophlevitits, systemic inflammatory response syndrome, thuroiditis, systemic lupus erythematosus, graft versus host disease, acute burn injury, Behcet's syndrome, and Sjogren's syndrome.

[0368] In another embodiment, the methods and compositions of the present invention encompass the treatment of the inflammatory diseases chosen from atherosclerosis, sepsis, asthma, autoimmune diseases, osteoporosis, rheumatoid arthritis, and osteoarthritis.

[0369] In another embodiment, the methods and compositions of the present invention encompass the treatment of muscular dystrophy and frailty.

[0370] In another embodiment, the present invention provides a method for the treatment of a neoplasia disorder in a subject, comprising administering to the subject a therapeutically effective amount of a liquid pharmaceutical composition comprising a human IgG2 antibody; and a pharmaceutically acceptable excipient comprising a chelating agent alone or in combination with other excipients chosen from a buffer,

an antioxidant, a tonicity agent, or a surfactant, and mixtures thereof. In further embodiments, the aforementioned subject is one that is in need of the treatment of a neoplasia disorder.

[0371] The terms, "neoplasia", "neoplasia diseases" and "neoplasia disorders", refer to a "neoplasm" or tumor, which may be benign, premalignant, metastatic, or malignant. Also encompassed by the present invention are benign, premalignant, metastatic, or malignant neoplasias. Also encompassed by the present invention are benign, premalignant, metastatic, or malignant tumors. Thus, all of benign, premalignant, metastatic, or malignant neoplasia or tumors are encompassed by the present invention and may be referred to interchangeably, as neoplasia, neoplasms or neoplasia-related conditions. Tumors are generally known in the art to be a mass of neoplasia or "neoplastic" cells. Although, it is to be understood that even one neoplastic cell is considered, for purposes of the present invention to be a neoplasm or alternatively, neoplasia.

[0372] Neoplasia disorders that may be treated by an anti-M-CSF antibody of the invention can involve any tissue or organ, and include, but are not limited to bone, brain, lung, squamous cell, bladder, gastric, pancreatic, breast, head, neck, liver, renal, ovarian, prostate, colorectal, esophageal, gynecological (e.g., cervical and ovarian), nasopharynx, or thyroid cancers. Also encompassed by the term neoplasia disorders, are bone metastases, melanomas, lymphomas, leukemias, and multiple myelomas. In particular, the anti-M-CSF antibody compositions of the present invention are useful to treat cancers of the breast, prostate, colon and lung.

[0373] In other embodiments, the methods and compositions of the present invention encompass the prevention and treatment of the neoplasia disorders selected from the group consisting of acral lentiginous melanoma, actinic keratoses, adenocarcinoma, adenoid cycstic carcinoma, adenomas, familial adenomatous polyposis, familial polyps, colon polyps, polyps, adenosarcoma, adenosquamous carcinoma, adrenocortical carcinoma, AIDS-related lymphoma, anal cancer, astrocytic tumors, bartholin gland carcinoma, -basal cell carcinoma, bile duct cancer, bladder cancer, brain stem glioma, brain tumors, breast cancer, bronchial gland carcinomas, capillary carcinoma, carcinoids, carcinoma, carcinoma of the fallopian tubes, carcinoma of the endometrium, carcinosarcoma, cavernous, central nervous system lymphoma, cerebral astrocytoma, cholangiocarcinoma, chondosarcoma, choriod plexus papilloma/carcinoma, clear cell carcinoma, skin cancer, brain cancer, colon cancer, colorectal cancer, cutaneous T-cell lymphoma, cystadenoma, endodermal sinus tumor, endometrial hyperplasia, endometrial stromal sarcoma, endometrioid adenocarcinoma, ependymal, epitheloid, esophageal cancer, Ewing's sarcoma, extragonadal germ cell tumor, fibrolamellar, focal nodular hyperplasia, gallbladder cancer, gastrinoma, germ cell tumors, gestational trophoblastic tumor, glioblastoma, glioma, glucagonoma, hemangiblastomas, hemangioendothelioma, hemangiomas, hepatic adenoma, hepatic adenomatosis, hepatocellular carcinoma, Hodgkin's lymphoma, hypopharyngeal cancer, hypothalamic and visual pathway glioma, insulinoma, intaepithelial neoplasia, interepithelial squamous cell neoplasia, intraocular melanoma, invasive squamous cell carcinoma, large cell carcinoma, islet cell carcinoma, Kaposi's sarcoma, kidney cancer, laryngeal cancer, leiomyosarcoma, lentigo maligna melanomas, leukemia-related conditions, lip and oral cavity cancer, liver cancer, lung cancer, lymphoma, malignant mesothelial tumors, malignant thymoma, medulloblastoma, medulloepithelioma, melanoma, meningeal, merkel cell carcinoma, mesothelial, metastatic carcinoma, mucoepidermoid carcinoma, multiple myeloma/plasma cell neoplasm, mycosis fungoides, myelodysplastic syndrome, myeloproliferative conditions, nasal cavity and paranasal sinus cancer, nasopharyngeal cancer, neuroblastoma, neuroepithelial adenocarcinoma nodular melanoma, neoplasms of the central nervous system (e.g., primary CNS lymphoma, spinal axis tumors, brain stem gliomas or pituitary adenomas), non-Hodgkin's lymphoma, oat cell carcinoma, oligodendroglial, oral cancer, oropharyngeal cancer, osteosarcoma, pancreatic polypeptide, ovarian cancer, ovarian, germ cell tumor, pancreatic cancer, papillary serous adenocarcinoma, pineal cell, pituitary tumors, plasmacytoma, pseudosarcoma, pulmonary blastoma, parathyroid cancer, penile cancer, pheochromocytoma, pineal and supratentorial primitive neuroectodermal tumors, pituitary tumor, plasma cell neoplasm, pleuropulmonary blastoma, prostate cancer, rectal cancer, renal cell carcinoma, retinoblastoma, rhabdomyosarcoma, sarcoma, serous carcinoma, small cell carcinoma, small intestine cancer, soft tissue carcinomas, somatostatin-secreting tumor, squamous carcinoma, squamous cell carcinoma, submesothelial, superficial spreading melanoma, supratentorial primitive neuroectodermal tumors, thyroid cancer, undifferentiated carcinoma, urethral cancer, uterine cancer, uveal melanoma, verrucous carcinoma, vaginal cancer, vipoma, vulvar cancer, Waldenstrom's macroglobulinemia, well differentiated carcinoma, and Wilm's tumor.

[0374] In a more preferred embodiment, the human IgG2 antibody is administered to a subject with breast cancer, prostate cancer, lung cancer or colon cancer. In an even more preferred embodiment, the method causes the cancer to stop proliferating abnormally, or not to increase in weight or volume or to decrease in weight or volume.

Articles of Manufacture

[0375] In another embodiment of the invention, an article of manufacture is provided comprising a container, which holds the composition comprising at least one of the monoclonal human IgG2 antibodies of the present invention in combination with a pharmaceutically acceptable chelating agent, and optionally provides instructions for its use. Suitable containers include, for example, bottles, bags, vials and syringes. The container may be formed from a variety of materials such as glass or plastic. An exemplary container is a 3-20 cc single use glass vial. Alternatively, for a multidose composition, the container may be 3-100 cc glass vial. The container holds the composition and the label on, or associated with, the container may indicate directions for use. The article of manufacture may further include other materials desirable from a commercial and user standpoint, including other buffers, diluents, filters, needles, syringes, and package inserts with instructions for use, contraindications, and/or lists of potential side-effects.

[0376] The present invention also provides a kit for preparing a liquid pharmaceutical composition of an antibody comprising: a first container comprising at least one human IgG2 antibody in solution, and a second container comprising a pharmaceutically acceptable chelating agent.

[0377] The following examples describe embodiments of the invention. Other embodiments within the scope of the claims herein will be apparent to one skilled in the art from consideration of the specification or practice of the invention as disclosed herein. It is intended that the specification, together with the examples, be considered exemplary only,

with the scope and spirit of the invention being indicated by the claims, which follow the examples. In the examples, all percentages are given on a weight basis unless otherwise indicated. The skilled artisan will appreciate that the weight quantities and/or weight-to-volume ratios recited in the examples can be converted to moles and/or molarities using the art-recognized molecular weights of the recited ingredients. Weight quantities exemplified herein (e.g., grams) are for the volumes (e.g., of buffer solutions, antibody composition, etc.) recited. The skilled artisan will appreciate that the weight quantities can be proportionally adjusted when different composition volumes are desired.

EXAMPLE 1-A

[0378] This Example shows the generation of hybridoma cell lines that produce anti-M-CSF antibodies as described in U.S. Published Application No. 20050059113 to Bedian, et al.,

Immunization and Hybridoma Generation

[0379] Eight to ten week old XENOMOUSE™ mice were immunized intraperitoneally or in their hind footpads with human M-CSF (10 μg/dose/mouse). This dose was repeated five to seven times over a three to eight week period. Four days before fusion, the mice were given a final injection of human M-CSF in phosphate buffered saline (PBS). The spleen and lymph node lymphocytes from immunized mice were fused with the non-secretory myeloma P3-X63-Ag8. 653 cell line, and the fused cells were subjected to HAT selection. See Galfre, G. and Milstein, C., "Preparation of monoclonal antibodies: strategies and procedures." *Methods Enzymol.* 73:3-46 (1981). A panel of hybridomas all secreting M-CSF specific human IgG2 and IgG4 antibodies was recovered. Antibodies also were generated using XENOMAX technology as described in Babcook, J. S. et al., *Proc. Natl. Acad. Sci. USA* 93:7843-48, 1996. Nine cell lines engineered to produce antibodies of the invention were selected for further study and designated 252, 88, 100, 3.8.3, 2.7.3, 1.120.1, 9.14.4, 8.10.3 and 9.7.2. The hybridomas were deposited under terms in accordance with the Budapest Treaty with the American Type Culture Collection (ATCC), 10801 University Blvd., Manassas, Va. 20110-2209 on Aug. 8, 2003. The hybridomas were assigned the following accession numbers:

Hybridoma 3.8.3 (LN 15891)	PTA-5390
Hybridoma 2.7.3 (LN 15892)	PTA-5391
Hybridoma 1.120.1 (LN 15893)	PTA-5392
Hybridoma 9.7.2 (LN 15894)	PTA-5393
Hybridoma 9.14.4 (LN 15895)	PTA-5394
Hybridoma 8.10.3 (LN 15896)	PTA-5395
Hybridoma 88-gamma (UC 25489)	PTA-5396
Hybridoma 88-kappa (UC 25490)	PTA-5397
Hybridoma 100-gamma (UC 25491)	PTA-5398
Hybridoma 100-kappa (UC 25492)	PTA-5399
Hybridoma 252-gamma (UC 25493)	PTA-5400
Hybridoma 252-kappa (UC 25494)	PTA-5401

EXAMPLE 2-A

[0380] This Example shows the generation of a recombinant mammalian cell line that produces anti-M-CSF antibodies.

[0381] DNA encoding the heavy and light chains of monoclonal antibodies 8.10.3 was cloned from the respective hybridoma cell line 8.10.3 and the DNA sequences were determined by methods known to one skilled in the art. The DNA from the hybridoma cell line 8.10.3 was mutated at specific framework regions in the variable domain to obtain 8.10.3F. From nucleic acid sequence and predicted amino acid sequence of the antibody 8.10.3F, the identity of the gene usage for each antibody chain was determined by ("VBASE"). Table 5 sets forth the gene utilization of antibody 8.10.3F in accordance with the present invention:

TABLE 5

Heavy and Light Chain Human Gene Utilization and Sequences

	Heavy Chain				Light Chain		
Antibody	SEQ ID NO:	V_H	D_H	J_H	SEQ ID NO:	V_κ	J_κ
8.10.3F	1 (nucleic acid) 2 (amino acid)	3-48	1-26	4b	3 (nucleic acid) 4 (amino acid)	A27	4

[0382] Antibody 8.10.3F DNA sequence inserts were obtained from the hybridoma cell line and subcloned into expression vectors. The expression vectors were then transfected into a mouse myeloma (NSO) host cell line to generate a primary transfectant cell line producing anti-M-CSF antibodies having the heavy and light chain sequences of 8.10.3F. Finally, samples of the 8.10.3F antibody producing NSO, cell line were frozen and stored in liquid nitrogen.

EXAMPLE 3-A

[0383] This Example shows the production of anti-M-CSF 8.10.3F antibodies from the NSO cell line generated according to Example 2-A.

[0384] A vial of 8.10.3F subcloned NSO cells was removed from liquid nitrogen storage as described in Example 2-A. The frozen cells were thawed rapidly to 37° C. until the last ice crystal disappeared. The entire contents (1 milliliter) of the thawed vial were then pipetted into a 75 cm^2 T-Flask. Fourteen milliliters of prewarmed (36.5° C.±1.0° C.) CD Hybridoma growth medium (available from Invitrogen, Carlsbad, Calif.) containing 10% Low IgG containing fetal bovine serum (available from Invitrogen, Carlsbad, Calif.) was slowly pipetted into the T-Flask.

[0385] The flask was planted at a target viable cell density of from about 2.0×10^5 to about 5.0×10^5 cells/ml. The flask was then placed in an incubator having a carbon dioxide level of 9% and a temperature of 36.5° C. and the cells were grown for about 3 days. At the end of this period, targeted cell number was on the order of 1.0 to 3.0×10^6 cells/ml.

[0386] After the cells were grown for about 3 days, they were split so that a target cell density of 2.5×10^5+/−0.5×10^5 was achieved and then disposable shake flasks (i.e., seed flasks) were seeded based on cell density. Each shake flask contained CD Hybridoma growth media containing 10% Low IgG containing fetal bovine serum. The flasks were then shaken at 100+/−10 rpm at 36.5° C.±1.0° C. for about 3 days. Cell density in each flask at the end of this period was 1.0 to 3.0×10^6 cells/ml and greater than 80% of the cells were viable.

[0387] After the cells were grown for about 3 days, the broth was harvested. Clarified broth was obtained after centrifugation for 15 minutes at 7000 rpm and subsequent filtration with a sterile 0.22 μm 4 inch Opticap™ Millipore™ filter into a sterile TC-Tech™ bag.

EXAMPLE 4-A

[0388] This Example shows the purification of anti-M-CSF antibodies from Example 3-A.

[0389] The clarified broth was then purified with three chromatographic steps comprising a Protein A affinity column and two ion exchange columns. A low pH inactivation and a viral filtration were also done to clear any potential viruses in the process. The product is concentrated and diafiltered into the composition buffer to make the anti-M-CSF antibody composition.

[0390] The Protein A column (Amersham Pharmacia) was prepped by washing with 3 column volumes of 8 M urea, followed by an equilibration wash with 20 mM Tris (pH 8). The final filtrate from Example 3 was spiked with 2% v/v of 1M Tris pH 8.3 and 0.02% NaN_3 before being loaded onto the Protein A column via gravity-drip mode. After load was complete, the resin was washed with 5 column volumes of 20 mM Tris (pH 8), followed by 5 column volumes of the elution buffer (0.1 M Glycine, pH 3.0). Any precipitation was noted, and then a 10% v/v spike of 1M Tris pH 8.3 was added to the eluted antibody. The eluted protein was then dialyzed into 100 fold the volume amount of eluted material of dialysis buffer (e.g., 140 mM sodium chloride/20 mM sodium acetate, pH 5.5). Following dialysis, the antibody was sterile filtered with a 0.22 μm filter and stored until further use.

EXAMPLE 5-A

[0391] A study was conducted to evaluate the effect of EDTA and histidine on discoloration, aggregation, and fragmentation in liquid compositions comprising monoclonal anti-M-CSF antibody 8.10.3F. Discoloration and aggregation in such liquid compositions are generally undesirable from a product aesthetic perspective, a product integrity perspective, or both.

Preparation of the Composition

[0392] The pharmaceutical compositions of the invention were made according to the following protocol. Materials which were used in preparation of the compositions include: glacial acetic acid 99.9% (Molecular Weight (MW) 60.05); concentrated sodium hydroxide 18.94N (50% w/w; MW 40.0); concentrated hydrochloric acid 37.8% (12.44N; MW 36.46); histidine (MW 155.16); sodium chloride (MW 58.44); mannitol (MW 182.17); polysorbate 80 (Crillet® 4 HP); sodium acetate trihydrate (MW 136.08); sodium citrate dihydrate (MW 294.1); disodium ethylenediaminetetraacetic acid dihydrate (MW 292.2); succinic acid (MW 118.1); antibody 8.10.3F bulk solution (about 10 mg/ml in sodium acetate, pH 5.5, prepared according to Examples 2-4); and water for injection (Milli-Q water). These solutions were prepared and then sterile filtered into 1 L Nalgene bottles and stored at 5° C.

[0393] The antibody compositions that were evaluated are listed in Table 6 below. To prepare each compositions, first the compositions buffers were made either with buffer only or with buffer and additional excipients such as tonicity agents (except addition of surfactant such as polysorbate 80) (reported in Table 6), followed by adjustment of pH to desired level. The buffer solutions were then filtered through sterilizing filter (0.22 micron pore size) into a sterilized receptacle. An antibody bulk solution from the purification process described in Example 4-A was obtained at about 10-15 mg/mL in 20 mM sodium acetate buffer pH 5.5 and 140 mM sodium chloride. Buffer exchanges of this bulk solution into the above identified composition solutions were carried out with Amicon® Centrifugal concentrators (e.g. with 30 kD cut-off membrane) on an Eppendorf 5810R centrifuge run at about 4500×g. At least 8 volume exchanges were made for each composition in respective buffers. Approximately 2 to 5 milliliters of compositions 1-A through 13-A were prepared. Antibody concentrations were determined by Ultraviolet-Visible spectrometry (UV-Vis) method using an extinction coefficient of 1.43 (mg/ml), 1 cm^{-1} at 280 nm. The final volume of the antibody solution was adjusted by appropriate dilution to achieve desired antibody concentration. A 20 mg/ml polysorbate 80 (PS80) solution was prepared by dilution and dissolution of polysorbate 80 by the appropriate composition buffer prepared as described above. For compositions 9-A to 13-A, addition of required quantity of 20 g/L polysorbate 80 solution was made to achieve 0.2 g/L polysorbate 80 in the antibody composition. The compositions with all of its ingredients included, was then sterilized by filtration through sterile 0.22 micron membrane filter.

[0394] For the composition number II-A (i.e., histidine, mannitol, polysorbate 80, and EDTA), a 1 molar (M) hydrochloric acid solution was first prepared by appropriate dilution from concentrated hydrochloric acid with water for injections. Individual solutions were then prepared by dissolving the following pre-weighed ingredients in about 90% of the water for injections: 45 grams per liter (g/L) of mannitol, 1.55 g/L of histidine, 0.02 g/L of disodium ethylenediaminetetraacetic acid dihydrate. After addition of all of the excipients except polysorbate 80, dissolution was achieved, and the pH of the solution was adjusted to pH 6 with 1M hydrochloric acid solution which was prepared as described above. After the addition of the hydrochloric acid solution, the final quantity of the water was added. The buffer solution was then filtered through a sterilization filter (0.22 micron pore size) into a sterilized receptacle.

[0395] A 20 g/L polysorbate 80 solution was prepared by appropriate dilution of polysorbate 80 by composition buffer (45 g/L of mannitol, 1.55 g/L of histidine, 0.02 g/L of disodium ethylenediaminetetraacetic acid dihydrate, pH 6).

[0396] The filtered compositions were then filled into vials. The vials were washed and autoclaved, as were the 13 mm Dalkyo 777-1 serum stoppers. A fill-volume of 0.25 to 1 ml was used in 2 ml Type 1 glass vials. The vials were closed with Dalkyo 777-1 Fluorotec® coated stoppers, crimp sealed, and placed in stability chambers.

Composition Appearance Analysis:

[0397] Each composition was visually evaluated at initial (i.e., time zero) and thereafter at desired sampling intervals (weeks) for particulate formation, color change, and turbidity change. Visual observations were reported in Table 6. The appearance assays were via visual inspection performed in a light box equipped with white and black backgrounds. Antibody concentrations were determined by ultraviolet-visible spectrometry (UV-Vis) methods using an extinction coefficient of 1.34 (mg/ml)-1.cm^{-1} at 280 nm.

TABLE 6

Description of Anti-M-CSF 8.10.3F Antibody Compositions, Appearance and Concentration.

Composition No.	Composition Description	pH	Visual Evaluation	8.10.3F Antibody concentration, (mg/ml)
1-A	20 mM sodium acetate	4.0	clear and colorless	8.2
2-A	5 mM sodium acetate, 5 mM sodium citrate, 5 mM histidine, 5 mM succinic acid	5.0	clear and colorless	11.9
3-A	5 mM sodium acetate, 5 mM sodium citrate, 5 mM histidine, 5 mM succinic acid	5.5	clear and colorless	8.2
4-A	20 mM histidine	6.0	clear and colorless	8.2
5-A	20 mM sodium citrate	5.5	clear and colorless	8.1
6-A	20 mM sodium acetate	5.5	clear and colorless	8.1
7-A	20 mM sodium succinate	5.5	clear and colorless	8.4
8-A	20 mM disodium EDTA dihydrate	5.5	clear and colorless	8.1
9-A	20 mM sodium acetate, 140 mM NaCl, 0.2 mg/ml polysorbate 80	5.5	clear and colorless	8.4
10-A	10 mM sodium acetate, 45 mg/ml Mannitol, 0.02 mg/ml EDTA, 0.2 mg/ml polysorbate 80	5.5	clear and colorless	9.0
11-A	10 mM histidine, 45 mg/ml mannitol, 0.02 mg/ml disodium EDTA dihydrate, 0.2 mg/ml polysorbate 80	6.0	clear and colorless	8.4
12-A	10 mM sodium citrate, 45 mg/ml mannitol, 0.02 mg/ml disodium EDTA dihydrate, 0.2 mg/ml polysorbate 80	5.5	clear and colorless	8.4
13-A	10 mM sodium succinate, 45 mg/ml mannitol, 0.02 mg/ml disodium EDTA dihydrate, 0.2 mg/ml polysorbate 80	5.5	clear and colorless	8.6

[0398] The results in Table 6 indicate that all tested antibody 8.3.1° F. compositions had no significant discoloration, no significant turbidity, and no significant particulate formation at the initial timepoint (i.e., time equal zero).

EXAMPLE 6-A

[0399] A study was conducted to evaluate the effect of various composition compositions and pH on anti-M-CSF antibody 8.10.3F fragmentation.

Fragmentation Analysis:

[0400] As noted above, the antibody compositions prepared according to Table 6 and Example 5-A were stored at a temperature of 40° C. At weeks, 0 (initial), 2, 4, and 6, the 40° C. compositions were analyzed for fragmentation using reduced SDS-PAGE (rSDS-PAGE). The composition vials were aseptically sampled at each time point and an aliquot from the vial was loaded onto NuPAGE 4-12% bis-Tris gels with colloidal blue (Coomassie) stain. Gel reduction was achieved by use of the NuPAGE® reducing agent. Percentage fragmentation (i.e., the presence of an 11 kilodalton (kD) polypeptide fragment and other fragments) in the reduced gels was estimated densitometrically by 100% minus (% heavy chain+% light chain) and reported in Table 7. FIG. **1** shows a line graph that shows the percent fragmentation (i.e., presence of polypeptides other than heavy chain (approx 50 kD) and light chain (approx 25 kD)) estimated from SDS-PAGE reduced gels. Reduced fragmentation was seen at pH ranges between 5.5 and 6.0. The gel data showed fragment bands with approximate molecular masses of 40 kD and 11 kD.

TABLE 7

Percent Fragmentation for Compositions in Table 6 after Storage at 40° C.:

Composition No.	Initial	2 weeks 40° C.	4 weeks 40° C.	6 weeks 40° C.
1-A	0	7%	20.1%	24.5%
2-A	0	5.3%	13.1%	18.1%
3-A	0	2.6%	6.6%	10.8%
4-A	0	2.5%	10.9%	12%
5-A	0	2.4%	4.7%	2.2%
6-A	0	0	2.2%	2.6%
7-A	0	0	6.2%	5.5%
8-A	0	2.7%	8.8%	9.4%
9-A	0	—	—	4.1%
10-A	0	—	—	2.9%
11-A	0	—	—	5.0%
12-A	0	—	—	4.6%
13-A	0	—	—	3.7%

[0401] FIG. **1** shows the percent fragmentation (i.e., presence of polypeptides other than heavy chain (about 50 kD) and light chain (about 25 kD) for each of the sample compo-

sitions detailed in Table 7. Reduced levels of fragmentation were seen in compositions having pH ranges between 5.5 and 6.0. Reduced levels of fragmentation were also seen in compositions without acetate, but having a chelating agent.

EXAMPLE 7-A

[0402] A study was conducted to evaluate the effect of various composition compositions and pH on anti-M-CSF antibody 8.10.3F charged species generation. Percentage major isoelectric focusing (IEF) band estimated from IEF gels with antibody samples stored at 40° C. over 6 weeks.

Formation of Acidic and Basic Species:

[0403] Antibody compositions 1-A through 4-A prepared according to Table 6 and Example 5-A were stored at a temperature of 40° C. After storing for 6 weeks, each composition was analyzed for the formation of acidic and basic species using Isoelectric Focusing (IEF). The Imaging Capillary Electrophoresis was conducted using a Convergent Biosciences iCE_{280} analyzer for evaluation of charge heterogeneity. The Convergent iCE_{280} is an imaging capillary isoelectric focusing (IEF) instrument, which allows the user to take an image of a separated sample contained within a capillary. IEF assays were conducted using pH 3-10.5 polyacrylamide gels and Coomassie blue stain. The sample protein components were separated based on their relative isoelectric points (pl). The major species was assigned based on the highest densitometric band intensity at a particular pl, in the initial samples. The change in percentage major species was followed as a function of storage duration. The loss in percentage major species from the initial value is a measure of the extent of acidic and basic specied formation.

[0404] Formation of acidic and basic species was also monitored by Imaging Capillary Electrophoresis (iCE). ICE was conducted using a Convergent Biosciences iCE_{280} analyzer for evaluation of charge heterogeneity. The Convergent iCE_{280} is an imaging capillary isoelectric focusing instrument, which allows the user to take an image of a separated sample contained within a capillary. The samples were prepared in a mixture of electrophoretic ampholytes, methyl cellulose, calibration markers, and water. The samples were introduced into the iCE_{280} and a high potential/voltage was applied. The sample protein components were separated based on their relative isoelectric points (pl). The relative amount of each separated component was observed by an imaging CCD camera. The data was then processed and reported as loss of the main peak (i.e., formation of acidic and basic species) using conventional chromatography integration software.

[0405] FIG. **2** shows the percentage major IEF band estimated from IEF gels with compositions 1-4 stored at 40° C. over 6 weeks. As seen in FIG. **2**, a lesser extent of decrease in the major IEF band at pH 5.5 and 6.0 suggested improved stability at pH ranging from 5.5 and 6.0 (i.e., composition nos. 3-A and 4-A).

EXAMPLE 8-A

[0406] A study was conducted to evaluate the effect of EDTA on anti-M-CSF antibody 8.10.3F aggregation.

[0407] Specifically, sample composition nos. 3-A, 5-A, 6-A, 7-A, and 8-A were prepared with and without EDTA according to Table 6 and Example 5-A and stored in several glass vials at 40° C. for 0 (initial), 2, 4, and 6 weeks. The glass vials were then sampled aseptically to measure the level of antibody 8.11.3F aggregation in the compositions at the 0, 2, 4, and 6 week time points. In addition, composition II-A (with EDTA) was also prepared according to Table 6 and stored in several glass vials at 40° C. for 26 weeks.

Aggregation Analysis:

[0408] After weeks 0, 2, 4, and 6, each composition was analyzed for aggregation using size exclusion chromatography. The size exclusion chromatography (i.e., SE-HPLC) was carried out using a TSK gel G3000SWXL-G2000SWXL column, mobile phase 0.2 M sodium phosphate buffer at pH 7.0, a flow rate of 1 ml/min, and UV detection at 214 nm. Table 8 shows the percentage of eluted high molecular weight species (i.e., aggregates of anti-M-CSF antibody 8.11.3F) measured at the relevant times for each of the composition treatments. Aggregation levels were calculated by integrating the areas under the chromatogram peaks for each composition and reporting the integrated areas under the high molecular weight species peaks as a percentage of total peak area (see Table 8).

TABLE 8

Percent Aggregation for Compositions 3, 5, 6, 7, and 8 after Storage at 40° C.:

Composition No.	Initial	2 weeks 40° C.	4 weeks 40° C.	6 weeks 40° C.
3-A	0.2%	1.3%	1.9%	3.2%
5-A	0.2%	1.5%	2.2%	3.8%
6-A	0.2%	1.1%	1.9%	3%
7-A	0.2%	1.2%	—	2.9%
8-A	0.2%	1.1%	1.4%	2.6%

[0409] As can be seen in Table 8 and FIG. **3**, the EDTA containing composition (i.e., composition 8-A) showed lower levels of aggregation over time as compared to compositions without EDTA. FIG. **11** shows a size exclusion chromatogram for monoclonal anti-M-CSF antibody 8.10.3F stored in composition II-A for 26 weeks at 40° C.

EXAMPLE 9-A

[0410] A study was conducted to evaluate the effect of EDTA on anti-M-CSF antibody 8.10.3F aggregation and fragmentation.

[0411] Specifically, sample composition nos. 9-A, 10-A, II-A, 12-A, and 13-A were prepared with and without EDTA according to Table 6 and Example 5-A and stored in several glass vials at 40° C. for 0 (initial), 4, 6, 8, 12 and 26 weeks. The glass vials were then sampled aseptically to measure the level of antibody 8.11.3F aggregation in the compositions at the predetermined time points.

Aggregation Analysis:

[0412] At weeks 0, 4, 6, 8, 12, and 26, each composition was analyzed for aggregation using size exclusion chromatography. Size exclusion—high pressure liquid chromatography (SE-HPLC) was carried out using a TSK gel G3000SWXL-G2000SWXL column, mobile phase 0.2 M sodium phosphate buffer at pH 7.0, a flow rate of 1 ml/min, and UV detection at 214 nm. Table 9 shows the percentage of

eluted high molecular weight species (i.e., aggregates of anti-M-CSF antibody 8.11.3F) measured at the relevant times for each of the composition treatments. Aggregation levels were calculated by integrating the areas under the chromatogram peaks for each composition and reporting the integrated areas under the high molecular weight species peaks as a percentage of total peak area (see Table 9).

TABLE 9

Percent Aggregation for Compositions 9-A, 10-A, 11-A, 12-A, and 13-A after Storage at 40° C.:

Composition No.	Initial	4 weeks 40° C.	6 weeks 40° C.	8 weeks 40° C.	12 weeks 40° C.	26 weeks 40° C.
9-A	0.2%	2.0%	2.6%	3.9%	5.8%	11.6%
10-A	0.1%	0.9%	1.4%	1.6%	2.7%	4.7%
11-A	0.2%	1.0%	1.4%	1.8%	2.6%	4.9%
12-A	0.2%	1.8%	2.3%	2.9%	3.9%	7.0%
13-A	0.2%	1.4%	1.9%	2.4%	3.5%	6.2%

[0413] As can be seen in Table 9 and FIG. **4**, the EDTA containing compositions (i.e., compositions 10-A, 11-A, 12-A and 13-A) showed lower levels of aggregation over time as compared to the composition without EDTA (i.e., composition 9-A).

Fragmentation Analysis:

[0414] At weeks 0, 4, 6, 8, 12, and 26, composition nos. 9-A, 10-A, II-A, 12-A, and 13-A were also analyzed for fragmentation.

[0415] Organic size exclusion—high pressure liquid chromatography (SE-HPLC) was conducted on the samples at time points 0, 4, 6, 8, 12 and 26 weeks to determine the percent fragmentation for an approximately 11 kD fragment of the antibody. The samples were injected onto a TSK gel Super SW3000 size exclusion column, using an isocratic mobile phase of 40% acetonitrile+0.1% TFA at a flow rate of 0.50 mL/min and UV detection at 214 nm. The percentage of eluted species was determined by integrating area under peaks.

TABLE 10

Percent Fragmentation of an approximately 11 kD Fragment for Compositions 9-A, 10-A, 11-A, 12-A, and 13-A after Storage at 40° C.:

Composition No.	Initial	8 weeks 40° C.	12 weeks 40° C.	26 weeks 40° C.
9-A	0.1%	1.6%	2.3%	3.5%
10-A	0.1%	0.7%	0.9%	1.7%
11-A	0.1%	0.6%	0.8%	1.7%
12-A	0.1%	1.5%	2.1%	4.1%
13-A	0.1%	1.0%	1.4%	3.0%

[0416] As can be seen in Table 10 and FIG. **5**, the EDTA containing compositions (i.e., compositions 10-A, II-A, 12-A and 13-A) showed lower levels of fragmentation yielding an 11 kD fragment over time as compared to the composition without EDTA (i.e., composition 9-A). In addition, the histidine containing composition (i.e., composition II-A) showed lower levels of fragmentation yielding an 11 kD fragment over time as compared to the compositions without histidine (i.e., compositions 9-A, 12-A and 13-A).

[0417] In addition, SDS-PAGE gels were also run with the samples at time points 0, 4, 6, 8, 12 and 26 weeks using NuPAGE 4-12% Bis-Tris gel, and colloidal blue (Coomassie) stain. For the reduced gels, reduction was achieved by NuPAGE® reducing agent. Percentage fragmentation in reduced gels was estimated densitometrically by 100% minus (% heavy chain+% light chain). The gel data showed fragment bands with approximate molecular masses of 40 kD and 11 kD. FIG. **6** shows the percentage fragmentation estimated from SBS-PAGE reduced gel data with the composition samples. FIG. **7** shows the percentage monomer of antibody estimated from SDS-PAGE non-reduced gel data with the composition samples.

[0418] FIGS. **4-7** show improved anti-M-CSF antibody stability for composition II-A (10 mM histidine, 45 mg/ml mannitol, 0.02 mg/ml disodium EDTA dihydrate, and 0.2 mg/ml polysorbate 80) for reduced aggregation (FIG. **4**), reduced quantity of 11 kD fragmentation (FIG. **5**), reduced fragmented species (FIG. **6**), and retaining highest % intact antibody monomer (FIG. **7**) as compared to compositions 9-A, 10-A, 12-A and 13-A.

EXAMPLE 10-A

[0419] A study was conducted to evaluate the effect of EDTA and histidine on anti-M-CSF antibody 8.10.3F fragmentation.

[0420] Several experimental compositions of antibody 8.10.3F generated truncated species (i.e., fragments) upon stressed conditions of 40° C. for 6 weeks, as observed by the formation of bands at approximately 40 kD and 11 kD appearing on reduced SDS-PAGE gel photograph as shown in FIG. **9**. The identity of the most abundant clip site was determined to be between residues Asp99 and Pro100 on the heavy chain of the molecule. Also observed were some minor truncation sites in the light chain, one between residues Gly213 and Glu214 and another between Glu214 and Cys215. The truncation level varies depending on the composition and thus far has only been observed at higher temperatures (e.g., 40° C.) i.e., under stressed conditions.

[0421] A sample of antibody 8.10.3F, which was formulated in sodium acetate and sodium chloride (composition no. 9-A) and stored at 40° C. for 26 weeks, was observed to have a higher presence of an 11 kD fragment than a composition comprising histidine and EDTA (composition no. II-A) using organic SE-HPLC (see FIG. **8**).

[0422] This sample was then analyzed by organic size exclusion chromatography/mass spectrometry (SEC/MS) in order to determine the site of truncation. The sample was injected onto a size exclusion column (Phenomenex SEC3000, 4.6×250 mm) using an isocratic mobile phase of 40% acetonitrile+0.1% TFA at a flow rate of 0.50 mL/min. The eluent of the column was split such that approximately half of the flow was directed into the source of an electrospray mass spectrometer (Micromass Q-T of Micro™, Waters Inc.). Mass spectra of each of the chromatographic peaks were deconvoluted using the MaxEnt algorithm included in the operating software. The measured molecular masses were then compared to the theoretical molecular mass based on the predicted amino acid sequence of antibody 8.10.3F.

[0423] Organic SE-HPLC separation with 214 nm detection followed by mass spectrometric identification were performed for antibody 8.10.3F in composition no. 1-A stored at

40° C. for 6 weeks compared to a control sample. FIG. **10** shows the resulting chromatogram having the 40° C. storage as the top graph and the 5° C. control as the bottom graph. The chromatogram measured masses were tabulated and compared to the theoretical masses of the postulated species in Table 11.

TABLE 11

The measured masses of the species observed in the sample compared with the theoretical masses of the postulated species. All of the measured molecular weights were within experimental error of the theoretical molecular weights of the postulated species.

Peak RT (retention time) (minutes)	Measured MW (Da)	Identity	Theoretical MW (Da)
4.45	147,220	8.10.3F ("Parent")	147,223*
4.45	136,424	Parent minus (Heavy Chain 1-99)	136,431*
4.45	125,634	Parent minus clipped 2x (Heavy Chain 1-99)	125,633*
6.53	10,816	Heavy chain 1-99	10,816

*Consistent with "G0, G0" glycoform and des-Lys C-terminus on heavy chains. The "G0, G1"; "G1, G1"; and "G1, G2" glycoforms were also observed. The "G0, G0" glycoform is a species in which both heavy chains have the G0 glycan attached, as described in Jefferis et al., Biochem. J., 268, 529-537, (1990). The G1 and G2 glycans have one and two, respectively, galactose residues on the nonreducing end of the glycan. The antibodies were N-glycosylated at residue 297 of the heavy chain.

[0424] Under stressed conditions, antibody 8.10.3F can undergo cleavage and generate truncated species. The main cleavage site is consistent with cleavage of an Asp-Pro bond in the heavy chain of 8.10.3F, which would generate a 10,816 Da (i.e., about 11 kd) species along with the corresponding parent species minus one and two of the truncation product.

EXAMPLE 11-A

[0425] A study was conducted to evaluate the effect of various buffers on anti-M-CSF antibody 8.10.3F aggregation.

[0426] Specifically, sample composition nos. 6-A, 3-A, 5-A and 8-A were prepared with according to Table 11 and Example 5-A and stored in glass vials at 40° C. for 6 weeks. The glass vials were then sampled aseptically to measure the level of antibody 8.11.3F aggregation in the compositions at the 6 week time point.

Aggregation Analysis:

[0427] At the 6 week time point, each composition was analyzed for aggregation using size exclusion chromatography. The size exclusion chromatography (i.e., SE-HPLC) was carried out using a TSK gel G3000SWXL-G2000SWXL column, mobile phase 0.2 M sodium phosphate buffer at pH 7.0, a flow rate of 1 ml/min, and UV detection at 214 nm. Table 11 shows the percentage of eluted high molecular weight species (i.e., aggregates of anti-M-CSF antibody 8.11.3F) measured at the relevant time for each of the composition treatments. Aggregation levels were calculated by integrating the areas under the chromatogram peaks for each composition and reporting the integrated areas under the high molecular weight species peaks that eluted prior to the antibody monomer (i.e., the intact unaggregated polypeptide) as a percentage of total peak area (see Table 11).

TABLE 12

Percent Aggregation for Compositions 6-A, 3-A, 5-A and 8-A after Storage at 40° C.:

Composition No.	8.10.3F Antibody concentration, (mg/ml)	Composition Description	% Aggregation
6-A	8 ± 2	20 mM acetate pH 5.5	3.0%
3-A	8 ± 2	Combination, pH 5.5 (acetate 5 mM, citrate 5 mM, histidine 5 mM, succinate 5 mM)	3.2%
5-A	8 ± 2	20 mM citrate, pH 5.5	3.8%
8-A	8 ± 2	20 mM EDTA, pH 5.5	2.6%

[0428] As can be seen in Table 11, the EDTA containing composition (i.e., composition 8-A) showed reduced levels of aggregation as compared to the compositions without EDTA (i.e., compositions 6-A, 3-A and 5-A).

EXAMPLE 12-A

[0429] A study was conducted to evaluate the effect of various excipients on anti-M-CSF antibody 8.10.3F aggregation and fragmentation.

[0430] Specifically, sample composition nos. 18-A, 19-A, 20-A, 29-A, 30-A and 31-A were prepared according to Table 12 and Example 5-A and stored in glass vials at 40° C. for 6 weeks. The glass vials were then sampled aseptically to measure the level of antibody 8.11.3F aggregation and fragmentation in the compositions at the 6 week time point.

Aggregation Analysis:

[0431] At the 6 week time point, each composition was analyzed for aggregation using size exclusion chromatography. The size exclusion chromatography (i.e., SE-HPLC) was carried out using a TSK gel G3000SWXL-G2000SWXL column, mobile phase 0.2 M sodium phosphate buffer at pH 7.0, a flow rate of 1 ml/min, and UV detection at 214 nm. Table 12 shows the percentage of eluted high molecular weight species (i.e., aggregates of anti-M-CSF antibody 8.11.3F) measured at the relevant time for each of the composition treatments. Aggregation levels were calculated by integrating the areas under the chromatogram peaks for each composition and reporting the integrated areas under the high molecular weight species peaks that eluted prior to the antibody monomer (i.e., the intact unaggregated polypeptide) as a percentage of total peak area (see Table 12).

Fragmentation Analysis:

[0432] At the 6 week time point, each composition was also analyzed for fragmentation using organic SE-HPLC. Organic SE-HPLC was conducted on the samples to determine the percent fragmentation for an 11 kD fragment of the total polypeptide. The samples were injected onto a TSK gel Super SW3000 size exclusion column, using an isocratic mobile phase of 40% acetonitrile+0.1% TFA at a flow rate of 0.50 mL/min and UV detection at 214 nm. The percentage of eluted species was determined by integrating area under peaks and reported in Table 12.

TABLE 12

Description of Anti-M-CSF 8.10.3F Antibody Compositions and Results after Storage at 40° C.:

Composition No.	8.10.3F mg/ml	Buffer	Excipient	PS80	$Na_2EDTA•2H_2O$	% Aggregate	% Fragment
29-A	6 ± 2	Sodium acetate, 20 mM, pH 5.5	Sodium chloride 140 mM	0.2	—	2.0%	1.2%
18-A	6 ± 2	Sodium acetate, 20 mM, pH 5.5	Mannitol 45 mg/ml	0.2	—	1.4%	1.0%
30-A	6 ± 2	Sodium acetate, 20 mM, pH 5.5	Mannitol 45 mg/ml	0.2	0.02 mg/ml	1.3%	0.6%
19-A	6 ± 2	Sodium acetate, 20 mM, pH 5.5	Sucrose 90 mg/mL	0.2	0.02 mg/ml	1.2%	0.5%
20-A	6 ± 2	Sodium acetate, 20 mM, pH 5.5	Trehalose 90 mg/mL	0.2	0.02 mg/ml	1.1%	0.4%
31-A	6 ± 2	Histidine, 10 mM, pH 6.0	Mannitol 45 mg/ml	0.2	0.02 mg/ml	0.8%	0.5%

[0433] As can be seen in Table 12, the EDTA containing compositions (i.e., compositions 30-A, 31-A, 19-A, and 20-A) showed reduced levels of aggregation and fragmentation as compared to the compositions without EDTA (i.e., compositions 29-A and 18-A).

EXAMPLE 13-A

[0434] A study was conducted to evaluate the effect of various excipients on anti-M-CSF antibody 8.10.3F fragmentation.

[0435] Specifically, sample composition nos. 21-A through 28-A were prepared according to Table 13 and Example 5-A and stored in glass vials at 40° C. for 26 weeks. The glass vials were then sampled aseptically to measure the level of antibody 8.11.3F fragmentation in the compositions at the 26 week time point.

Fragmentation Analysis:

[0436] At the 26 week time point, each composition was also analyzed for fragmentation using organic SE-HPLC. Organic SE-HPLC was conducted on the samples to determine the percent fragmentation for an 11 kD fragment of the total polypeptide. The samples were injected onto a TSK gel Super SW3000 size exclusion column, using an isocratic mobile phase of 40% acetonitrile+0.1% TFA at a flow rate of 0.50 mL/min and UV detection at 214 nm. The percentage of eluted species was determined by integrating area under peaks and reported in Table 13.

TABLE 13

Descripition of Anti-M-CSF 8.10.3F Antibody Compositions and Results after Storage at 40° C.:

Composition No.	8.10.3F mg/ml	Buffer	Excipients	PS80 mg/mL	$Na_2EDTA•2H_2O$ mg/mL	% Fragmentation
21-A	50 ± 8	Sodium acetate, 20 mM, pH 5.5	Sodium chloride 140 mM	0.2	—	4.1%
22-A	50 ± 8	Sodium acetate, 10 mM, pH 5.5	Mannitol 45 mg/ml	0.2	0.02 mg/ml	2.3%
23-A	50 ± 8	Sodium acetate, 10 mM, pH 5.5	Sucrose 90 mg/ml	0.2	0.02 mg/ml	3.0%
24-A	50 ± 8	Sodium acetate, 10 mM, pH 5.5	Trehalose 90 mg/ml	0.2	0.02 mg/ml	2.2%

TABLE 13-continued

Descripition of Anti-M-CSF 8.10.3F Antibody Compositions and Results after Storage at 40° C.:

Composition No.	8.10.3F mg/ml	Buffer	Excipients	PS80 mg/mL	$Na_2EDTA•2H_2O$ mg/mL	% Fragmentation
25-A	50 ± 8	Histidine, 10 mM, pH 6.0	Mannitol 45 mg/ml	0.2	0.02 mg/ml	2.1%
26-A	50 ± 8	Histidine, 10 mM, pH 6.0	Sucrose 90 mg/ml	0.2	0.02 mg/ml	1.9%
27-A	50 ± 8	Histidine, 10 mM, pH 6.0	Trehalose 90 mg/ml	0.2	0.02 mg/ml	1.8%
28-A	50 ± 8	Histidine, 5 mM, pH 6.0	Mannitol 45 mg/ml	0.2	0.02 mg/ml	1.4%

[0437] As can be seen in Table 13, the EDTA containing compositions (i.e., compositions 22-A through 28-A) showed reduced levels of fragmentation as compared to the composition without EDTA (i.e., composition 21-A). Likewise, as can be seen in Table 14, the histidine containing compositions (i.e., compositions 25-A through 28-A) showed reduced levels of fragmentation as compared to the compositions without histidine (i.e., compositions 21-A through 24-A).

EXAMPLE 1-B

[0438] This Example shows the generation of hybridoma cell lines that produce anti-CTLA-4 antibodies as described in U.S. Pat. No. 6,682,736 to Hanson, et al.,

[0439] Antibodies of the invention were prepared, selected, and assayed as follows:

[0440] Antigen Preparation: Three distinct immunogens were prepared for immunization of the XenoMouse™ mice: (i) a CTLA-4-IgG fusion protein, (ii) a CTLA-4 peptide, and (iii) 300.19 murine lymphoma cells transfected with a mutant of CTLA-4 (Y201V) that is constitutively expressed on the cell surface.

CTLA-4-IgG1 Fusion Protein:

Expression Vector Construction

[0441] The cDNA encoding the mature extracellular domain of CTLA-4 was PCR amplified from human thymus cDNA library (Clontech) using primers designed to the published sequence (*Eur. J Immunol* 18:1901-1905 (1988)). The fragment was directionally subcloned into pSR5, a Sindbis virus expression plasmid (InVitrogen), between the human oncostatin M signal peptide and human IgG gamma 1 (IgG1) CH1/CH2/CH3 domains. The fusion protein does not contain a hinge domain but contains cysteine 120 in the extracellular domain of CTLA-4 to form a covalent dimer. The resulting vector was called CTLA-4-IgG1/pSR5. The complete CTLA-4-IgG1 cDNA in the vector was sequence confirmed in both strands. The amino acid sequence the CTLA4-1 g protein is shown below. The mature extracellular domain for CD44 was PCR amplified from human lymphocyte library (Clontech) and subcloned into pSinRep5 to generate a control protein with the identical IgG1 tail.

OM-CTLA4-IgG1 Fusion Protein:

[0442]

MGVLLTQRTLLSLVLALLFPSMASMAMHVAQPAVVLASSRGIASFVCEYA

SPGKATEVRVTVLRQADSQVTEVCAATYMMGNELTFLDDSICTGTSSGNQ

VNLTIQGLRAMDTGLYICKVELMYPPPYYLGIGNGTQIYVIDPEPCPDSD

LEGAPSVFLFPPKPKDTLMISRTPEVTCVVVDVSHEDPEVKFNWYVDGVE

VHNAKTKPREEQYNSTYRVVSVLTVLHQDWLNGKEYKCKVSNKALPTPEE

KTISKAKGQPREPQVYTLPPSRDELTKNQVSLTCLVKGFYPSDIAVEWES

NGQPENNYKTTPPVLDSDGSFFLYSKLTVDKSRWQQGNVFSCSVMHEALH

NHYTQKSLSLSPGK

Underlined = signal peptide

[0443] The cDNAs for mature extracellular domain of CD28 were PCR amplified from human lymphocyte library (Clontech) and then subcloned into pCDM8 (*J. Immunol.* 151: 5261-71 (1993)) to produce a human IgG1 fusion protein containing both thrombin cleavage and hinge regions. Marmoset, Cynomologous, and Rhesus CTLA4 were cloned from mRNA isolated from PHA stimulated PBMCs using standard techniques of degenerate PCR. Sequencing demonstrated that rhesus and cynomologous amino acid sequence were identical with three differences from mature human CTLA4 extracellular domain (S13N, I17T and L105M). Marmoset demonstrated ten amino acid differences from the mature human CTLA4 extracellular domain (V21A, V33I, A41T, A51G, 541, S71F, Q75K, T88M, L105M and G106S). Site directed mutagenesis was used to make single point mutations of all amino acids different in marmoset CTLA4 to map amino acids important for interation of the antibodies with human CTLA4-IgG. Mutations of human and marmoset CTLA-IgG for epitope mapping were generated by matchmaker site-directed mutagenesis (Promega). The IgG fusion proteins were produced by transient transfection of Cos7 cells and purified using standard Protein A techniques. Mutant CTLA4-IgG proteins were evaluated for binding to antibodies by immunoblotting and using BIAcore analyses.

Recombinant Protein Expression/Purification

[0444] Recombinant sindbis virus was generated by electroporating (Gibco) Baby Hamster Kidney cells with SP6 in

vitro transcribed CTLA-4-IgG1/pSR5 mRNA and DH-26S helper mRNA as described by InVitrogen. Forty eight hours later recombinant virus was harvested and titered for optimal protein expression in Chinese hamster ovary cells (CHO-K1). CHO-K1 cells were cultured in suspension in DMEM/F12 (Gibco) containing 10% heat-inactivated fetal bovine serum (Gibco), non-essential amino acids (Gibco), 4 mM glutamine (Gibco), penicillin/streptomycin (Gibco), 10 mM Hepes pH 7.5 (Gibco). To produce CTLA-4-IgG, the CHO-K1 cells were resuspended at 1×10^7 cells/ml in DMEM/F12 and incubated with sindbis virus for one hour at room temperature. Cells were then diluted to 1×10^6/ml in DMEM/F12 containing 1% fetal bovine serum depleted of bovine IgG using Protein A Sepharose (Pharmacia), non-essential amino acids, 4 mM glutamine, 12.5 mM Hepes pH 7.5, and penicillin/streptomycin. Forty eight hours post-infection cells were pelleted and conditioned media was harvested and supplemented with complete protease inhibitor tablets (Boehringer Mannheim), pH adjusted to 7.5, and filtered 0.2μ, (Nalgene). FPLC (Pharmacia) was used to affinity purify the fusion protein using a 5 ml protein A HiTrap column (Pharmacia) at a 10 ml/min flow rate. The column was washed with 30 bed volumes of PBS and eluted with 0.1M glycine/HCl pH 2.8 at 1 ml/min. Fractions (1 ml) were immediately neutralized to pH 7.5 with Tris pH 9. The fractions containing CTLA-4-IgG1 were identified by SDS-PAGE and then concentrated using centriplus 50 (Amicon) before applying to sepharose 200 column (Pharmacia) at 1 ml/min using PBS as the solvent. Fractions containing CTLA-4-IgG1 were pooled, sterile filtered 0.2μ. (Millipore), aliquoted and frozen at −80°. CD44-IgG1 was expressed and purified using the same methods. CD28-IgG was purified from conditioned media from transiently transfected Cos7 cells.

Characterization CTLA-4-IgG1:

[0445] The purified CTLA-4-IgG1 migrated as a single band on SDS-PAGE using colloidal coomassie staining (Novex). Under non-reducing conditions CTLA-4-IgG1 was a dimer (100 kDa), that reduced to a 50 kDa monomer when treated with 50 mM DTT. Amino acid sequencing of the purified CTLA-4-IgG1 in solution confirmed the N-terminus of CTLA-4 (MHVAQPAVVLAS) and that the oncostatin-M signal peptide was cleaved from the mature fusion protein. The CTLA-4-IgG1 bound to immobilized B7.1-IgG in a concentration dependent manner and the binding was blocked by a hamster-anti-human anti-CTLA-4 antibody (BNI3: PharMingen). The sterile CTLA-4-IgG was endotoxin free and quantitated by OD280 using 1.4 as the extinction coefficient. The yield of purified CTLA-4-IgG ranged between 0.5-3 mgs/liter of CHO-K1 cells.

CTLA-4 Peptide:

[0446] The following CTLA-4 peptide was prepared as described below: NH_2:MHVAQPAVVLASSRGIASFVCEYASPGKATEVRVTVLRQADSQVTEVCMTYMMGNELTF LDDSICTGTSSGNQ VNLTIQGLRAMDTGLYICKVELMYPPPYYLGIGNGTQIYVIDPEPCCONH$_2$

Abbreviations/Materials:

[0447] NMP, N-Methylpyrrolidinone; TFE, 2,2,2-Trifluoroethanol; DCM, Dichloromethane; FMOC, Fluorenyl Methoxycarbonyl. All reagents were supplied by Perkin Elmer, with the following exceptions: TFE, Aldrich Chemical, FMOC-PAL-PEG resin, Perseptive Biosystems. Fmoc-Arg (PMC)-OH; FMOC-Asn(Trt)-OH, FMOC-Asp(tBu)-OH, FMOC-Cys(Trt)-OH, FMOC-Glu(tBu)-OH, FMOC-Gln (Trt)-OH, FMOC-His(Boc)-OH, FMOC-Lys(BOC)-OH, FMOC-Ser(tBu)-OH, FMOC-Thr(tBu)-OH and FMOC-Tyr (tBu)-OH were used for those amino acids requiring side chain protecting groups.

Peptide Synthesis:

[0448] Peptide synthesis was performed on a Perkin-Elmer 431A, retrofitted with feedback monitoring via UV absorbance at 301 nm (Perkin-Elmer Model 759A detector). The peptide sequence was assembled on a FMOC-PAL-PEG resin using conditional double coupling cycles. Forced double couplings were performed at cycles 10, 11, 18, 19, 20 and 28 through 33. The resin was washed with a 50% mixture of DCM and TFE at the completion of each acylation cycle, followed by capping of unreacted amino groups with acetic anhydride in NMP Resin was removed from the reactor after completing cycle 49 and the remainder continued to completion. Peptide cleavage from the resin was performed using Reagent K (King et al. International Journal of Protein and Peptide Research 36:255-266 (1990)) for 6 hours on 415 mg of resin affording 186 mg crude CTLA-4 peptide.

Peptide Characterization:

[0449] 25 mg aliquots of the crude CTLA-4 peptide were dissolved in 5 ml 6M Guanidine HCl/100 mM K_2PO_3 at pH6.4 and eluted over a Pharmacia Hi Load Superdex 75 16/60 column (16 mm×600 mm, 120 ml bed volume) with 2M Guanidine HCl/100 mM K_2PO_3 at pH 6.4 at 2 ml/min for 180 minutes collecting 5 ml fractions. The fractions were analyzed by loading 1.7 μl of fractions onto a NuPAGE Laemeli gel running with MES running buffer and visualizing via Daichii silver stain protocol. Those fractions exhibiting a molecular weight of 12 KDa, as judged versus molecular weight standards, were pooled together and stored at 4° C. The combined fractions were analyzed by UV and gel electrophoresis. Amino acid sequencing was performed by absorbing a 100 microliter sample in a ProSorb cartridge (absorbed onto a PVDF membrane) and washing to remove the buffer salts. Sequencing was performed on an Applied Biosystems 420 sequencer. The expected N-terminal sequence (MHVAQPAWLA) was observed. Immunoblotting demonstrated that the peptide was recognized by the BNI3 anti-human CTLA-4 antibody (PharMingen). To desalt, an aliquot containing 648 μg of material was placed in 3500 Da MWCO dialysis tubing and dialyzed against 0.1% TFA/H20 at 4° C. for 9 days with stirring. The entire contents of the dialysis bag was lyophilyzed to a powder.

"300.19" Cells Transfected with CTLA-4 (Y201V) Peptide Antigen:

[0450] The full length CTLA-4 cDNA was PCR amplified from human thymus cDNA library (Stratagene) and subcloned into pIRESneo (Clontech). A mutation of CTLA-4 that results in constitutive cell surface expression was introduced using MatchMaker Mutagenesis System (Promega). Mutation of tyrosine, Y201 to valine inhibits binding of the adaptin protein AP50 that is responsible for the rapid internalization of CTLA-4 (Chuang, et al. *J. Immunol.* 159:144-151 (1997)). Mycoplasma-free 300.19 murine lymphoma cells were cultured in RPMI-1640 containing 10% fetal calf

serum, non-essential amino acids, penicillin/streptomycin, 2 mM glutamine, 12.5 mM Hepes pH 7.5, and 25 μM beta-mercaptoethanol. Cells were electroporated (3×10^6/0.4 ml serum free RPMI) in a 1 ml chamber with 20 ug CTLA-4-Y201V/pIRESneo using 200V/1180 uF (Gibco CellPorator). Cells were rested for 10 minutes and then 8 mls of prewarmed complete RPMI media. At 48 hours cells were diluted to 0.5×10^6/ml in complete RPMI media containing 1 mg/ml G418 (Gibco). Resistant cells were expanded and shown to express CTLA-4 on the cell surface using the BNI3 antibody conjugated with phycoerythrin (PharMingen). High level expressing cells were isolated by sterile sorting.

Immunization and Hybridoma Generation:

[0451] XenoMouse™ mice (8 to 10 weeks old) were immunized (i) subcutaneously at the base of tails with 1×10^7 300.19 cells that were transfected to express CTLA-4 as described above, resuspended in phosphate buffered saline (PBS) with complete Freund's adjuvant, or (ii) subcutaneously at the base of tail with (a) 10 μg the CTLA-4 fusion protein or (b) 10 μg CTLA-4 peptide, emulsified with complete Freund's adjuvant. In each case, the dose was repeated three or four times in incomplete Freund's adjuvant. Four days before fusion, the mice received a final injection of the immunogen or cells in PBS. Spleen and/or lymph node lymphocytes from immunized mice were fused with the [murine non-secretory myeloma P3 cell line] and were subjected to HAT selection as previously described (Galfre, G. and Milstein, C., "Preparation of monoclonal antibodies: strategies and procedures." *Methods Enzymol.* 73:3-46 (1981)). A large panel of hybridomas all secreting CTLA-4 specific human IgG_2K antibodies were recovered.

[0452] The following hybridoma producing anti-CTLA-4 antibodies designated as follows were deposited at the American Type Culture Collection, 10801 University Blvd. Manassas, Va. 20110-2209, on Apr. 29, 2003:

Clone	Subclone	ATCC Deposit No.
11.2.1	11.2.1.4	PTA-5169
4.1.1	4.1.1.1	PTA-5166

EXAMPLE 2-B

[0453] This Example shows the generation of recombinant mammalian cell lines that produce anti-CTLA-4 antibodies.

[0454] DNA encoding the heavy and light chains of monoclonal antibody 11.2.1 was cloned from the respective hybridoma cell line 11.2.1 and the DNA sequences were determined by methods known to one skilled in the art. From nucleic acid sequence and predicted amino acid sequence of the antibody 11.2.1, the identity of the gene usage for each antibody chain was determined.

[0455] The 11.2.1 DNA sequence inserts were then subcloned into expression vectors. The expression vectors were subsequently transfected into a mouse myeloma (NSO) host cell to generate various primary transfectant cell lines that produce anti-CTLA antibodies. A lead cell line was chosen based on growth and productivity analysis. The lead line was later sub-cloned to generate a clonal cell line.

[0456] The anti-CTLA4 antibody was produced by cell cultivation using the cell line in a bioreactor containing cell culture media. The media is supplement with nutrients during production. After harvest criteria were attained, the bioreactor was harvested either by filtration alone or by centrifugation followed by filtration. The clarified supernatant was then purified with three chromatographic steps comprising a Protein A affinity column and two ion exchange columns. A low pH inactivation and a viral filtration were also done to clear any potential viruses in the process. The product is concentrated and diafiltered into the composition buffer to make the drug substance.

EXAMPLE 3-B

[0457] A study was conducted to evaluate the effect of four different buffers on antibody aggregation and fragmentation.

[0458] Specifically, four liquid compositions comprising anti-CTLA4 antibody 11.2.1 and buffered with acetate, succinate, histidine or EDTA were prepared. The compositions then were stored at 40° C. and antibody aggregation and fragmentation measurements were taken at 0, 2, 5 and 7 weeks.

Preparation of Buffer Solutions:

[0459] Four buffer solutions were prepared as described in Table 13. Each solution was prepared by first dissolving an amount of the buffer species (listed in Table 13) in water (approximately 80% of target). The pH of each buffer solution was then adjusted to 5.5 by addition of a sufficient amount of the acid or base solution noted in Table 13. After adjustment of the pH, an additional amount of water was added to provide a final buffer concentration of 20 mM. The buffer concentration of 20 mM was selected to ensure reasonable pH stability at the selected pH of 5.5. The buffer solution was then filtered through a sterilization filter (0.22 micron pore size) into a sterilized receptacle for subsequent use.

TABLE 13

Buffer Solutions:

Buffer Type	Buffer Species	Buffer Concentration (g/L)	Acid/Base Solution
Acetate	Sodium acetate trihydrate	2.74	1% v/v Glacial Acetic Acid
Succinate	Succinic acid	2.36	1 M NaOH
Histidine	L-Histidine HCl monohydrate	4.19	1 M NaOH
EDTA	Disodium EDTA dihydrate	7.45	5 M HCl

[0460] The 1% v/v glacial acetic acid solution was prepared by appropriate dilution (1 ml to 100 ml) of glacial acetic acid (99.9%) with water. The 1 molar (M) sodium hydroxide solution was prepared by dissolving 40 g of solid sodium hydroxide in 1 L of water. The 5 molar (M) hydrochloric acid solution was prepared by appropriate dilution of concentrated hydrochloric acid (37.8%) with water.

Preparation of Antibody Compositions:

[0461] The antibody compositions that were evaluated are listed in Table 14 below. To prepare each composition, an amount of the tonicifier (reported in mg/ml in Table 14) was first added to the indicated buffer solution and the solution stirred until the tonicifier dissolved. An antibody bulk solution from the purification process described in Example 2-B

was obtained at 13.2 mg/mL in 20 mM sodium acetate buffer pH 5.5+140 mM sodium chloride. Buffer exchanges of this bulk solution into the above identified composition solutions were carried out with Amicon Ultra 15 MWCO10K (UFC901024) Centrifugal concentrators on a Beckman Coulter Allegra 21R Centrifuge run at 6500 RPM at 5° C. Approximately 8 volume exchanges were made and the antibody solution concentrated to between 27 and 30 mg/ml. Approximately 3 to 4 mls of compositions 1-B through 18-B were prepared. Antibody concentrations were determined by Ultraviolet-Visible spectrometry (UV-Vis) method using an extinction coefficient of 1.43 $(mg/ml)^{-1}$ cm^{-1} at 280 nm.

[0462] A 20 mg/ml polysorbate 80 (PS80) solution was prepared by dilution and dissolution of polysorbate 80 by the appropriate composition buffer prepared as described above. The polysorbate 80 was then added to the antibody and buffer solutions as a 20 mg/ml concentrate along with appropriate amount of buffer, antibody, tonicifier and water to obtain a 20 mg/ml final solution of the anti-CTLA-4 monoclonal antibody in the composition corresponding to the compositions in Table 4 below.

[0463] For Composition No. 2-B in Table 14, the PEG3350 was added as a 200 mg/ml concentrate at this point.

[0464] The compositions were then filtered through 0.2μ sterilizing grade filters and filled into vials. A fill-volume of 0.5 to 1 ml was used in 2 ml Type 1 glass vials. The vials were closed with Dalkyo 777-1 Fluorotec® coated stoppers, crimp sealed, and placed in stability chambers stored upright at 40° C. for 2, 5 and 7 weeks. The vials were washed and autoclaved, as were the 13 mm Dalkyo 777-1 serum stoppers. Duplicate vials were immediately analyzed for levels of aggregation and fragmentation.

TABLE 14

Antibody Compositions Tested:

Composition No.	Buffer Type 20 mM, pH 5.5	Tonicifier (mg/ml)	Surfactant (mg/ml)
1-B	Acetate	NaCl (9)	PS80 (0.2)
2-B	Acetate	NaCl (9) + PEG3350 (10)	PS80 (0.2)
3-B	Acetate	Sucrose (90)	PS80 (0.2)
4-B	Acetate	Sorbitol (48)	PS80 (0.2)
5-B	Acetate	Inositol (48)	PS80 (0.2)
6-B	Acetate	Mannitol (41) + Glycine (2)	PS80 (0.2)
7-B	Succinate	Sucrose (90)	PS80 (0.2)
8-B	Succinate	Sorbitol (48)	PS80 (0.2)
9-B	Succinate	Inositol (48)	PS80 (0.2)
10-B	Succinate	Mannitol (41) + Glycine (2)	PS80 (0.2)
11-B	Histidine	Sucrose (90)	PS80 (0.2)
12-B	Histidine	Sorbitol (48)	PS80 (0.2)
13-B	Histidine	Inositol (48)	PS80 (0.2)
14-B	Histidine	Mannitol (41) + Glycine (2)	PS80 (0.2)
15-B	EDTA	Sucrose (90)	PS80 (0.2)
16-B	EDTA	Sorbitol (48)	PS80 (0.2)
17-B	EDTA	Inositol (48)	PS80 (0.2)
18-B	EDTA	Mannitol (41) + Glycine (2)	PS80 (0.2)

[0465] Aggregation Analysis:

[0466] The antibody compositions of Table 14 were stored at a temperature of 40° C. At weeks 0, 2, 5 and 7, each composition was analyzed for aggregation using size exclusion chromatography (SEC). The size exclusion chromatography was carried out using a TSK gel G3000SWXL-G2000SWXL column, mobile phase 0.2 M sodium phosphate buffer at pH 7.0, a flow rate of 1 ml/min, and UV detection at 214 nm. FIG. **13** shows the percentage of eluted high molecular weight species (i.e., aggregates of anti-CTLA-4 monoclonal antibody 11.2.1) measured at the relevant times for each of the compositions. Aggregation levels were calculated by integrating the areas under the chromatogram peaks for each composition and reporting the integrated areas under the high molecular weight species peaks as a percentage of total peak area (see FIG. **13**). As can be seen in FIG. **13**, the EDTA-buffered compositions showed the lowest levels of aggregation, followed by the histidine-, acetate-, and succinate-buffered compositions, in that order.

Fragmentation Analysis:

[0467] As noted above, the antibody compositions of Table 14 were stored at a temperature of 40° C. At weeks 0, 2, 5 and 7, each composition also was analyzed for fragmentation using rSDS-PAGE. The rSDS-PAGE analysis was carried out using NuPAGE 4 to 12% bis-Tris gel and colloidal blue (Coomassie) stain. For the reduced gels (rSDS-PAGE), reduction was achieved by NuPAGE® reducing agent. Total hydrolytic impurities (i.e., fragments of anti-CTLA-4 monoclonal antibody 11.2.1) were estimated by scanning using either a Molecular Dynamics Personal Densitometer PDQC-90 or a Bio-Rad GS800 Imaging Densitometer. FIG. **14** shows the percentage of fragmentation measured at the relevant times for each of the compositions. The fragmentation levels were calculated as a percentage of total band volume (see FIG. **14**). As can be seen in FIG. **14**, the EDTA-buffered compositions showed the lowest levels of fragmentation, followed by the histidine-, acetate-, and succinate-buffered compositions, in that order.

[0468] Table 15(a) (0 weeks), Table 15(b) (2 weeks), Table 15(c) (5 weeks), and Table 15(d) (7 weeks) below report the aggregation and fragmentation data that is graphically presented in FIGS. **13** and **14**.

TABLE 15(a)

Aggregation and Fragmentation Results at 0 Time Point:

Composition No.	Percent Aggregation	Percent Fragmentation
1-B	0.4%	0.62%
2-B	0.4%	0.66%
3-B	0.3%	0.53%
4-B	0.4%	0.49%
5-B	0.4%	0.67%
6-B	0.3%	0.56%
7-B	0.3%	0.46%
8-B	0.4%	0.62%
9-B	0.4%	0.49%
10-B	0.3%	0.51%
11-B	0.3%	0.64%
12-B	0.3%	0.62%
13-B	0.3%	0.47%
14-B	0.3%	0.37%
15-B	0.3%	0.42%
16-B	0.3%	0.50%
17-B	0.3%	0.49%
18-B	0.3%	0.47%

TABLE 15(b)

Aggregation and Fragmentation Results at 2 Week Time Point:

Composition No.	Percent Aggregation	Percent Fragmentation
1-B	0.7%	1.52%
2-B	0.7%	1.35%

TABLE 15(b)-continued

Aggregation and Fragmentation Results at 2 Week Time Point:

Composition No.	Percent Aggregation	Percent Fragmentation
3-B	0.5%	1.16%
4-B	0.5%	1.13%
5-B	0.5%	1.10%
6-B	0.4%	1.34%
7-B	0.6%	1.34%
8-B	0.6%	1.44%
9-B	0.6%	1.22%
10-B	0.5%	1.16%
11-B	0.4%	1.29%
12-B	0.4%	1.19%
13-B	0.4%	1.00%
14-B	0.4%	0.99%
15-B	0.5%	1.24%
16-B	0.5%	1.00%
17-B	0.5%	1.07%
18-B	0.5%	0.96%

TABLE 15(c)

Aggregation and Fragmentation Results at 5 Week Time Point:

Composition No.	Percent Aggregation	Percent Fragmentation
1-B	0.8%	1.40%
2-B	0.9%	1.59%
3-B	1.2%	2.61%
4-B	1.1%	1.49%
5-B	1.0%	2.12%
6-B	0.6%	1.56%
7-B	1.7%	2.50%
8-B	1.4%	1.86%
9-B	1.4%	2.03%
10-B	0.9%	1.46%
11-B	0.6%	1.42%
12-B	0.7%	1.36%
13-B	0.6%	1.03%
14-B	0.5%	1.05%
15-B	0.5%	1.21%
16-B	0.5%	0.78%
17-B	0.6%	1.27%
18-B	0.5%	1.25%

TABLE 15(d)

Aggregation and Fragmentation Results at 7 Week Time Point:

Composition No.	Percent Aggregation	Percent Fragmentation
1-B	1.4%	2.39%
2-B	1.2%	1.90%
3-B	1.4%	1.89%
4-B	1.8%	2.96%
5-B	1.3%	1.92%
6-B	1.1%	1.77%
7-B	1.2%	1.97%
8-B	2.2%	2.91%
9-B	2.4%	3.25%
10-B	1.3%	1.82%
11-B	0.9%	1.34%
12-B	0.9%	1.33%
13-B	0.7%	1.12%
14-B	0.6%	0.92%

TABLE 15(d)-continued

Aggregation and Fragmentation Results at 7 Week Time Point:

Composition No.	Percent Aggregation	Percent Fragmentation
15-B	0.6%	1.04%
16-B	0.6%	1.18%
17-B	0.6%	1.01%
18-B	0.6%	1.18%

EXAMPLE 4-B

[0469] A study was conducted to evaluate the ability of different liquid compositions comprising monoclonal anti-CTLA-4 antibody 11.2.1 to tolerate multiple freezing and thawing cycles.

[0470] The ability of a liquid composition to withstand multiple freeze/thaw cycles is often evaluated to determine whether the composition may be stored (and, if desired, transported) frozen and then thawed for later use.

[0471] The compositions that were evaluated are listed in Table 16 below. The procedure used to prepare the compositions is the same as the one described in Example 3-B. 2.5 mL of each solution was placed in 5-mL type I glass vials, stoppered, and sealed. The compositions identified below as numbers 1-B to 4-B, 7-B to 8-B, 11-B to 12-B, and 15-B to 16-B were identical to the compositions having the same number identifiers in Example 3-B.

TABLE 16

Antibody Compositions Tested:

Composition No.	Buffer Type 20 mM, pH 5.5	Tonicifier (mg/ml)	Surfactant (mg/ml)
1-B	Acetate	NaCl (9)	PS80 (0.2)
2-B	Acetate	NaCl (9) + PEG3350 (10)	PS80 (0.2)
3-B	Acetate	Sucrose (90)	PS80 (0.2)
4-B	Acetate	Sorbitol (48)	PS80 (0.2)
19-B	Acetate	Trehalose (90)	PS80 (0.2)
20-B	Succinate	NaCl (9)	PS80 (0.2)
21-B	Succinate	NaCl (9) + PEG3350 (10)	PS80 (0.2)
7-B	Succinate	Sucrose (90)	PS80 (0.2)
8-B	Succinate	Sorbitol (48)	PS80 (0.2)
22-B	Histidine	NaCl (9)	PS80 (0.2)
23-B	Histidine	NaCl (9) + PEG3350 (10)	PS80 (0.2)
11-B	Histidine	Sucrose (90)	PS80 (0.2)
12-B	Histidine	Sorbitol (48)	PS80 (0.2)
24-B	EDTA	NaCl (9)	PS80 (0.2)
25-B	EDTA	NaCl (9) + PEG3350 (10)	PS80 (0.2)
15-B	EDTA	Sucrose (90)	PS80 (0.2)
16-B	EDTA	Sorbitol (48)	PS80 (0.2)

[0472] Each composition was subjected to six consecutive freeze/thaw cycles. The first three cycles were carried out in a controlled rate freezer. The last three cycles were slower cycles carried out with a number of water-filled vials to correspond to a high thermal load placed in a freezer or refrigerator. For cycles 1, 2 and 3, the vials containing the compositions were placed in a controlled rate freezer (Planer Kryo 560-16) and subjected to the following cycle: cool the composition at a rate of 0.2° C./min until a temperature of −70° C. is reached, hold at −70° C. for 1.5 to 3 hours, and thaw the

composition at a rate of 0.3° C./min until a temperature of 5° C. is reached. For cycles 4, 5 and 6, the vials were placed in a box along with other water-filled vials (one sample vial for each composition; 17 composition vials with a total of about 30 water-filed vials). This box was then placed first in a freezer maintained at a temperature of −70° C. freezer for approximately 17 hours, and then placed in a refrigerator maintained at a temperature of 2-8° C. for approximately 50 hours. A recording thermal probe placed in the box measured an average cooling rate of 0.09° C./min for the freeze process and an average heating rate of 0.03° C./min for the thaw process.

[0473] Each composition was visually evaluated after each freeze/thaw cycle for particulate formation, color change and turbidity change. Such visual observations of each composition were performed in a light box against black and white backgrounds while the composition was still cold after each thaw. Table 17 (below) reports the results.

TABLE 17

Visual Evaluations of Freeze/thaw Stability of Anti-CTLA-4 antibody 11.2.1

No.	ID	1xFT	2xFT	3xFT	4xFT	5xFT	6xFT	% Increase in Particulates (SEC)
1-B	Ac + NaCl	colorless, no particulates	colorless, no particulates	colorless, no particulates	turbid	lots of particulates	lots of particulates	0.7
2-B	Ac + NaCl + PEG	colorless, no particulates	colorless, no particulates	colorless, no particulates	flakes	cloudy with flakes	few particulates	0
3-B	Ac + Sucrose	colorless, no particulates	colorless, no particulates	colorless, no particulates	colorless, no particulates	colorless, no particulates	colorless, no particulates	0.1
4-B	Ac + Sorbitol	colorless, no particulates	colorless, no particulates	colorless, no particulates	colorless, no particulates	colorless, no particulates	colorless, no particulates	0.1
19-B	Ac + Trehalose	colorless, no particulates	colorless, no particulates	colorless, no particulates	colorless, no particulates	colorless, no particulates	colorless, no particulates	0
20-B	Succ + NaCl	few particulates	few particulates	more particulates	more particulates	cloudy, flakes, particulates	lots of particulates	1.3
21-B	Succ + NaCl + PEG	colorless, no particulates	fewer particulates	few particulates	cloudy	cloudy	few particulates	0.2
7-B	Succ + Sucrose	colorless, no particulates	colorless, no particulates	colorless, no particulates	colorless, no particulates	colorless, no particulates	colorless, no particulates	0.1
8-B	Succ + Sorbitol	colorless, no particulates	colorless, no particulates	colorless, no particulates	colorless, no particulates	colorless, no particulates	colorless, no particulates	0
22-B	Hist + NaCl	colorless, no particulates	turbid	turbid	cloudy, flakes	cloudy	cloudy, turbid, flakes	1.1
23-B	Hist + NaCl + PEG	colorless, no particulates	turbid	few particulates	colorless, no particulates	cloudy flakes, few particulates	few particulates	0.1
11-B	Hist + Sucrose	colorless, no particulates	colorless, no particulates	colorless, no particulates	colorless, no particulates	colorless, no particulates	colorless, no particulates	0.1
12-B	Hist + Sorbitol	colorless, no particulates	colorless, no particulates	colorless, no particulates	colorless, no particulates	colorless, no particulates	colorless, no particulates	0
24-B	Ed + NaCl	colorless, no particulates	colorless, no particulates	colorless, no particulates	cloudy, fewer particulars	lots of particulates	lots of particulates, cloudy	0.1
25-B	Ed + NaCl + PEG	colorless, no particulates	turbid, fewer particulates	cloudy, flakes	cloudy	cloudy, flakes	few particulates	0
15-B	Ed + Sucrose	colorless, no particulates	colorless, no particulates	colorless, no particulates	colorless, no particulates	colorless, no particulates	colorless, no particulates	0
16-B	Ed + Sorbitol	colorless, no particulates	colorless, no particulates	colorless, no particulates	colorless, no particulates	colorless, no particulates	colorless, no particulates	0.1

[0474] The compositions containing only sodium chloride (i.e., chloride ions) exhibited a greater increase in soluble particulate levels after freeze/thaw cycling than the compositions containing trehalose, sucrose or sorbitol. The addition of PEG to the compositions containing sodium chloride, however, appeared to reduce soluble particulate levels measured after freeze/thaw cycling relative to the corresponding compositions not containing PEG.

Aggregation Analysis:

[0475] In addition, the percent increase in soluble particulates was measured for each composition after 6 consecutive freeze/thaw cycles using size exclusion chromatography.

[0476] After the sixth freeze/thaw cycle, each composition was analyzed for aggregation using size exclusion chromatography. The size exclusion chromatography was carried out using a TSK gel G3000SWXL-G2000SWXL column, mobile phase 0.2 M sodium phosphate buffer at pH 7.0, a flow rate of 1 ml/min, and UV detection at 214 nm. Table 17 shows the percentage of eluted high molecular weight species (i.e., aggregates of anti-CTLA-4 monoclonal antibody 11.2.1) measured at the relevant times for each of the compositions. Aggregation levels were calculated by integrating the areas under the chromatogram peaks for each composition and reporting the integrated areas under the high molecular weight species peaks as a percentage of total peak area (see Table 17). As can be seen in Table 17, the EDTA-buffered compositions showed the lowest levels of aggregation, followed by the histidine-, acetate-, and succinate-buffered compositions, in that order.

EXAMPLE 5-B

[0477] A study was conducted to evaluate the effect of EDTA, methionine and anaerobic conditions on discoloration and aggregation in liquid compositions comprising monoclonal anti-CTLA-4 antibody 11.2.1. Discoloration and aggregation in such liquid compositions are generally undesirable from a product aesthetic perspective, a product integrity perspective, or both.

[0478] Table 8 below lists the composition treatments that were evaluated. The general procedure used to prepare the compositions was the same as the one described in Example 3. For this Example, a starting composition comprising monoclonal anti-CTLA-4 antibody 11.2.1 (5 mg/ml), a sodium acetate buffer (20 mM), sodium chloride (8.2 mg/ml), and polysorbate 80 (0.2 mg/ml) and having pH 5.5 was prepared and added to several 10-mL glass vials containing seal tops to allow for aseptic sampling.

[0479] Various treatments were performed on the starting composition according to Table 18 below. As noted in Table 18, methionine was added to some of the vials. Two different concentrations of EDTA were added to other vials. Nitrogen gas was added to the headspaces of selected EDTA- or methionine-containing vials. In addition, some of the remaining untreated vials were deaerated prior to injection of nitrogen gas into their headspaces. Further, some of the remaining vials were left untreated to act as experimental controls.

[0480] Two vials from each of the treatments in Table 18 were stored at 40° C. for 0, 2, 4, 6, 8, 10, 14, 16, and 18 weeks. One of the two stored vials at each time point was used for visual color evaluations while the other vial was sampled aseptically to measure the level of 11.2.1 antibody aggregation after storage. Tables 19 and 20 report the results.

TABLE 18

	Antibody Composition Treatments Tested:	
Composition No.	Treatment Identification	Treatment
26-B	No treatment	None
27-B	+N_2 gas in the headspace	Change head-space in vial to Nitrogen gas in lyophilizer by evacuation and replacement
28-B	+Deaerated + N_2 gas in headspace	De-aerated in lyophilizer, change head-space in vial to Nitrogen in lyophilizer as in #2 above
29-B	+26.6 mM Methionine	Added 26.6 mM Methinone as solid
30-B	+N_2 gas in the headspace + 26.6 mM Methionine	Added 26.6 mM Methionine as solid and changed head-space in vial to Nitrogen in lyophilizer as in #2 above
31-B	+0.005% Na_2EDTA	Added 0.005% $Na_2EDTA•2H_2O$ as solid
32-B	+26.6 mM Methionine + 0.005% Na_2EDTA	Added 26.6 mM Methionine and 0.005% $Na_2EDTA•2H_2O$ as solids
33-B	+0.01% Na_2EDTA	Added 0.01% $Na_2EDTA•2H_2O$ as solid

Composition Appearance Analysis:

[0481] Each composition was visually evaluated after 0 (initial), 2, 4, 6, 8, 10, 14, 16, and 18 weeks for particulate formation, color change and turbidity change. Visual observations were reported in Table 19.

TABLE 19

			Visual Evaluations after Composition Treatments in Table 18:								
No.	Vial Treatment	Initial	40° C. 2 weeks	40° C. 4 weeks	40° C. 6 weeks	40° C. 8 weeks	40° C. 10 weeks	40° C. 12 weeks	40° C. 14 weeks	40° C. 16 weeks	40° C. 18 weeks
26-B	No treatment	clear and colorless	clear and colorless	pink	pink	pink	pink	pink	pink	pink	pink
27-B	+N_2	clear and colorless	clear and colorless	pink	pink	pink	pink	pink	pink	pink	pink
28-B	+Deaerated + N_2	clear and colorless	clear and colorless	pink	pink	pink	pink	pink	pink	pink	pink
29-B	+26.6 mM Methionine	clear and colorless	clear and colorless	clear and colorless	clear and colorless	clear and colorless	clear and colorless	clear and colorless	clear and colorless	clear and colorless	clear and colorless
30-B	+N_2 + 26.6 mM Methionine	clear and colorless	clear and colorless	clear and colorless	clear and colorless	clear and colorless	clear and colorless	clear and colorless	clear and colorless	clear and colorless	clear and colorless
31-B	+0.005% Na_2EDTA	clear and colorless	clear and colorless	clear and colorless	clear and colorless	clear and colorless	clear and colorless	clear and colorless	clear and colorless	clear and colorless	clear and colorless
32-B	+26.6 mM Methionine + 0.005% Na_2EDTA	clear and colorless	clear and colorless	clear and colorless	clear and colorless	clear and colorless	clear and colorless	clear and colorless	clear and colorless	clear and colorless	clear and colorless
33-B	+0.01% Na_2EDTA	clear and colorless	clear and colorless	clear and colorless	clear and colorless	clear and colorless	clear and colorless	clear and colorless	clear and colorless	clear and colorless	clear and colorless

[0482] The results in Table 19 indicate that the compositions without EDTA and/or methionine developed a pink coloration in the vial after storage for at least 4 weeks at 40° C. While not wishing to be bound by any particular theory, it is believed that in one embodiment of the invention, this color change may be due, at least in part, to an oxidative process. However, in other embodiments, the color change may be due to any number of other processes, which are unrelated to oxidation.

[0483] Addition of Nitrogen gas to the headspace of the vials appeared to have less of an affect on reducing the discoloration than the addition of methionine and/or EDTA.

Aggregation Analysis:

[0484] The antibody compositions treated according to Table 18 were stored at a temperature of 40° C. At weeks 0, 2, 6, 8, 10, 14, 16, and 18, each composition was analyzed for aggregation using size exclusion chromatography. The size exclusion chromatography was carried out using a TSK gel G3000SWXL-G2000SWXL column, mobile phase 0.2 M sodium phosphate buffer at pH 7.0, a flow rate of 1 ml/min, and UV detection at 214 nm. Table 20 shows the percentage of eluted high molecular weight species (i.e., aggregates of anti-CTLA-4 antibody 11.2.1) measured at the relevant times for each of the composition treatments. Aggregation levels were calculated by integrating the areas under the chromatogram peaks for each composition and reporting the integrated areas under the high molecular weight species peaks as a percentage of total peak area (see Table 20).

TABLE 20

			Percent Aggregation for Composition Treatments in Table 18:								
No.	Vial Treatment	Initial	40° C. 2 weeks	40° C. 4 weeks	40° C. 6 weeks	40° C. 8 weeks	40° C. 10 weeks	40° C. 12 weeks	40° C. 14 weeks	40° C. 16 weeks	40° C. 18 weeks
26-B	No treatment	0.2%	0.8%	2.3%	3.5%	4.6%	5.31%	4.3%	8.8%	7.7%	7.1%
27-B	+N_2	0.2%	0.7%	2.3%	3.8%	4.4%	4.82%	4.5%	6.5%	6.0%	5.5%
28-B	+Deaerated + N_2	0.2%	0.3%	0.9%	1.4%	2.5%	3.68%	4.3%	—	4.6%	4.8%
29-B	+26.6 mM Methionine	0.2%	0.2%	0.5%	0.5%	0.6%	0.59%	0.5%	0.7%	0.8%	0.7%
30-B	+N_2 + 26.6 mM Methionine	0.2%	0.2%	0.5%	0.4%	0.4%	0.51%	0.5%	0.5%	0.7%	0.7%
31-B	+0.005% Na_2EDTA	0.2%	0.2%	0.4%	0.6%	0.5%	0.69%	0.8%	1.0%	1.0%	0.8%
32-B	+26.6 mM Methionine + 0.005% Na_2EDTA	0.2%	0.3%	0.3%	0.4%	0.4%	0.35%	0.4%	0.8%	0.5%	0.7%
33-B	+0.01% Na_2EDTA	0.2%	0.2%	0.4%	0.6%	0.6%	0.73%	0.3%	1.2%	1.0%	1.2%

[0485] The results in Table 19 indicate that the compositions without EDTA and/or methionine begin to develop a pink coloration in the vial after storage for at least 4 weeks at 40° C. As can be seen in Table 20, the EDTA and/or methionine treated compositions showed the lowest levels of aggregation, followed by the Nitrogen gas-treated and untreated control compositions.

EXAMPLE 6-B

[0486] A study was conducted to evaluate the effect of methionine and EDTA on the oxidation of certain methionine amino acid residues in the anti-CTLA-4 antibody 11.2.1 after storage as a liquid composition.

Methionine Oxidation Analysis:

[0487] Oxidation levels of methionine residues at amino acid positions 256 and 432 in anti-CTLA-4 antibody 11.2.1 were measured by a Lysine-C mapping method after storage for 8 weeks at 40° C.

[0488] Glass vials containing composition nos. 26-B, 29-B and 33-B (Table 18) and their treatments from Example 5-B were aseptically sampled at the 8 week time point. The samples were then digested with Lyc-C enzyme in tris buffer at pH 8.0 under standard conditions and analyzed by reversed-phase high performance liquid chromatography. Separation was accomplished using a Grace Vydac Protein C4 analytical column with 0.1% TFA in water and 0.085% TFA in Acetonitrile gradient elution.

TABLE 21

Percent Oxidation of Methionine Amino Acids in Anti-CTLA-4 antibody 11.2.1 After Treatments in Table 18:

Composition No.	Treatment	Percent Oxidation Met 432	Percent Oxidation Met 256
	Initial	2.3%	4.9%
26-B	No treatment	15.4%	32.9%
29-B	+26.6 mM Methionine	0.5%	1.1%
33-B	+0.01% Na_2EDTA	1.6%	3.3%

[0489] The results in Table 11 indicate that the addition of methionine or EDTA to the 11.2.1 antibody composition reduces the percent oxidation at the two indicated methionine residues as compared to the composition stored without EDTA or methionine.

EXAMPLE 7-B

[0490] A study was conducted to evaluate the oxidation of certain tryptophan and tyrosine amino acid residues in the anti-CTLA-4 antibody 11.2.1.

[0491] Anti-CTLA-4 antibody 11.2.1 compositions that develop a pink discoloration over time were found to have a characteristic absorption maximum at 500 nm after conducting ultraviolet/visible spectroscopy (UV-Vis).

[0492] The procedure used to prepare the composition is the same as the one described in Example 3-B. For this Example, a composition comprising a 5 mg/ml solution of monoclonal anti-CTLA-4 antibody 11.2.1 in a 20 mM sodium acetate buffer, 8.2 mg/ml sodium chloride and 0.2 mg/ml polysorbate 80 (at pH 5.5) was stored in two glass vials for 4 weeks at 40° C., at which time, the compositions had developed a pink discoloration.

[0493] The solution in one of the discolored vials composition was then subjected to molecular weight (cut-off) filtration, which allowed the composition excipients to pass through the filtration device, while leaving behind the antibodies. The filtration eluent (e.g., water and excipients) was clear and colorless, while the collected fraction (e.g., antibody 11.2.1) remained pink. Thus, the filtration experiment indicated that the pink discoloration was related to the antibody 11.2.1 itself in contrast to arising from the composition's excipients.

[0494] Next, the second vial having the pink discoloration was digested with trypsin under standard conditions and analyzed by reversed phase high performance liquid chromatography coupled with mass spectrometry (LC-MS). Separation was accomplished using a Grace Vydac Protein C4 analytical column with 0.1% TFA in water and 0.085% TFA in Acetonitrile gradient elution. The UV-Vis absorbance of the digested peptides at 500 nm was monitored, and the corresponding peptides were identified on the basis of their molecular weight.

[0495] The tryptic peptide, which correlates with the 500 nm absorbance peak, had the amino acid sequence: GLEWVAVIWYDGSNK. The peptide sequence GLEWVAVIWYDGSNK was then digested further with Asp-N protease under standard conditions, and the 500 nm absorbance (UV-Vis) peak migrated along with the Asp-N protease digested peptide, which had the amino acid sequence: GLEWVAVIWY.

[0496] Therefore, without intending to be bound by any particular theory, it is believed that either one or both of the two tryptophan amino acid residues (W) or the tyrosine residue (Y) within the protease digested peptide (GLEWVAVIWY) are possible sites for oxidation, which may have been responsible for the pink discoloration of the antibody 11.2.1 composition in this example. In particular embodiments, it is believed that either one or both of the two tryptophan amino acid residues (W) within the protease digested peptide (GLEWVAVIWY) are possible sites for oxidation, which may have been responsible for the pink discoloration.

[0497] Although, it is also possible that mechanisms other than oxidation may have been responsible for any one or more of the particular discolorations (e.g., pink and yellow) seen in the various compositions evaluated herein.

EXAMPLE 8-B

[0498] A study was conducted to evaluate the effect of EDTA and DTPA on anti-CTLA-4 antibody 11.2.1 discoloration, aggregation and fragmentation.

[0499] Specifically, three liquid compositions comprising antibody 11.2.1 with and without EDTA and DTPA were prepared. The compositions were stored at 40° C. and antibody discoloration, aggregation and fragmentation evaluations were conducted at 0, 2, 4, 6, 8 and 10 weeks.

[0500] For this Example, a 20 mg/ml solution of anti-CTLA-4 antibody in 20 mM sodium acetate buffer pH 5.5 with 8.2 mg/ml sodium chloride and 0.2 mg/ml polysorbate 80 was prepared and divided among several glass vials, as described in Example 3, and then treated by addition of EDTA or DTPA. The EDTA and DTPA were added to the composition vials as solids. Several vials were immediately analyzed for levels of discoloration, aggregation and fragmentation and several other duplicate vials were also stored upright at 40° C. for 2, 4, 6, 8 and 10 weeks.

[0501] The treated and untreated vials were then sampled aseptically to measure the level of antibody 11.2.1 aggregation and fragmentation in the compositions at the 2, 4, 6, 8 and 10 week time points and observed for discoloration. Tables 22 and 23 report the results.

Composition Appearance Analysis:

[0502] Each composition was visually evaluated after 0 (initial), 2, 4, 6, 8 and 10 weeks for particulate formation, color change and turbidity change. Visual observations were reported in Table 22.

TABLE 22

Visual Evaluations of EDTA and DTPA Composition Treatments:

No.	Treatment	0 weeks Initial	2 weeks 40° C.	4 weeks 40° C.	6 weeks 40° C.	8 weeks 40° C.	10 weeks 40° C.
26-B	No treatment	clear and colorless	clear and colorless	clear and colorless	pink	pink	pink
33-B	+0.01% $Na_2EDTA•2H_20$	clear and colorless	clear and colorless	clear and colorless	clear and colorless	clear and colorless	clear and colorless
34-B	+0.01% DTPA	clear and colorless	clear and colorless	clear and colorless	clear and colorless	clear and colorless	clear and colorless

[0503] The results in Table 22 indicate that the compositions without EDTA or DTPA developed a pink coloration in the vial after storage for at least 6 weeks at 40° C.

Aggregation Analysis:

[0504] The antibody compositions treated according to Table 22 were stored at a temperature of 40° C. At weeks 0, 2, 6, 8 and 10, each composition was analyzed for aggregation using size exclusion chromatography. The size exclusion chromatography was carried out using a TSK gel G3000SWXL-G2000SWXL column, mobile phase 0.2 M sodium phosphate buffer at pH 7.0, a flow rate of 1 ml/min, and UV detection at 214 nm. Table 23 shows the percentage of eluted high molecular weight species (i.e., aggregates of anti-CTLA-4 antibody 11.2.1) measured at the relevant times for each of the composition treatments. Aggregation levels were calculated by integrating the areas under the chromatogram peaks for each composition and reporting the integrated areas under the high molecular weight species peaks as a percentage of total peak area (see Table 23).

TABLE 23

Percent Aggregation for EDTA and DTPA Composition Treatments:

No.	Treatment	0 weeks Initial	2 weeks 40° C.	4 weeks 40° C.	6 weeks 40° C.	8 weeks 40° C.	10 weeks 40° C.
26-B	No treatment	0.6%	0.9%	1.4%	2.2%	2.6%	3.2%
33-B	+0.01% $Na_2EDTA•2H_20$	0.7%	0.8%	0.9%	0.9%	1.0%	1.2%
34-B	+0.01% DTPA	0.5%	0.7%	0.8%	0.9%	0.9%	0.9%

[0505] As can be seen in Table 23, both the EDTA and DTPA containing compositions showed lower levels of aggregation compared to the composition without EDTA or DTPA.

EXAMPLE 9-B

[0506] A study was conducted to evaluate the effect of EDTA and nitrogen gas on anti-CTLA-4 antibody 11.2.1 stability.

[0507] Specifically, the impact of EDTA and nitrogen gas on antibody 11.2.1 stability was analyzed with regards to discoloration, aggregation, oxidation, fragmentation and formation of charged species in histidine-buffered compositions containing trehalose and polysorbate 80.

[0508] The compositions that were evaluated are listed in Table 23 below. The procedure used to prepare the compositions is the same as the one described later in Example 10-B. The compositions were stored at 40° C. and stability evaluations were conducted at 0, 4, 8, 12 and 24 weeks.

[0509] For this Example, a 20 mg/ml solution of anti-CTLA-4 antibody in 20 mM histidine buffer at pH 5.5 with 84 mg/ml trehalose and 0.2 mg/ml polysorbate 80 was prepared as in Example 10-B. One part of the composition was prepared by diluting concentrated stock solution of the antibody with stock solutions of trehalose and Polysorbate 80 to the final composition at 20 mg/mL anti-CTLA-4 antibody. A second part of the composition was prepared similarly except for the additional step of addition of a 10 mg/mL concentrate of $Na_2EDTA.2H_2O$ to achieve a final concentration of 0.1 mg/mL. The compositions were then dispensed at 1 ml per 2 ml glass vials. Half of the vials of each composition were then placed in a lyophilizer, and the head-space 4 changed to nitrogen after evacuation. After the vials were charged with nitrogen, a measurement of their oxygen levels reported about 1.5% to 1.6% oxygen, while vials with air in the head-space reported about 19.7% to 20% oxygen.

[0510] Several vials were immediately analyzed for levels of discoloration, aggregation, fragmentation, oxidation, and formation of charged species and several other duplicate vials were also stored upright at 40° C. for 2, 4, 8, 12 and 24 weeks.

At each time point, two stored vials per treatment were removed from each condition to measure the level of antibody 11.2.1 aggregation, fragmentation, oxidation and formation of charged species in the compositions and observed for discoloration. Tables 24 through 28 report the results.

TABLE 23

Antibody Compositions Tested:

No.	Vial Head-Space	Buffer	Tonicifier	Surfactant	Chelating Agent
35-B	Air	Histidine	Trehalose	Polysorbate 80	—
36-B	Nitrogen	Histidine	Trehalose	Polysorbate 80	—
37-B	Air	Histidine	Trehalose	Polysorbate 80	EDTA
38-B	Nitrogen	Histidine	Trehalose	Polysorbate 80	EDTA

Composition Appearance Analysis:

[0511] Each composition was visually evaluated after 0 (initial), 4, 8, 12 and 24 weeks for particulate formation, color change and turbidity change. Visual observations were reported in Table 24.

TABLE 24

Visual Evaluations after Composition Treatments in Table 23:

No.	Treatment	0 weeks Initial	4 weeks 40° C.	8 weeks 40° C.	12 weeks 40° C.	24 weeks 40° C.
35-B	+Air in the headspace	clear, nb particulates	very slight pink, less than 3 particulates	very slight pink, no particulates	slight pink no particulates	slight pink, no particulates
36-B	+Nitrogen in the headspace	clear, no particulates	clear, no particulates	clear, no particulates	very slight pink, no particulates	very slight pink, no particulates
37-B	+Air in the headspace + EDTA	clear, no particulates	clear, no particulates	clear, less than 3 particulates	clear, no particulates	clear, no particulates
38-B	+Nitrogen in the headspace + EDTA	clear, no particulates	clear, no particulates	clear, no particulates	clear, no particulates	clear, no particulates

[0512] The results in Table 24 indicate that the composition without EDTA or nitrogen gas developed a pink coloration after storage for 4 weeks at 40° C. Table 24 also indicates that the composition having the vial headspace air replaced with nitrogen gas delayed the onset of pink discoloration until week 12. Both compositions containing EDTA had no visible discoloration for at least 24 weeks.

Aggregation Analysis:

[0513] The antibody compositions prepared according to Table 23 were stored at a temperature of 40° C. At weeks 0, 4, 8, 12 and 24, each composition was analyzed for aggregation using size exclusion chromatography. The composition vials were aseptically sampled at each time point. The size exclusion chromatography was carried out on the samples using a TSK gel G3000SWXL-G2000SWXL column, mobile phase 0.2 M sodium phosphate buffer at pH 7.0, a flow rate of 1 ml/min, and UV detection at 214 nm. Table 25 shows the percentage of eluted high molecular weight species (i.e., aggregates of anti-CTLA-4 antibody 11.2.1) measured at the relevant times for each of the composition treatments. Aggregation levels were calculated by integrating the areas under the chromatogram peaks for each composition and reporting the integrated areas under the high molecular weight species peaks as a percentage of total peak area (see Table 25).

TABLE 25

Percent Aggregation for Compositions in Table 23:

Composition No.	Treatment	0 weeks Initial	4 weeks 40° C.	8 weeks 40° C.	12 weeks 40° C.	24 weeks 40° C.
35-B	+Air in the headspace	0.7%	0.9%	1.3%	1.5%	3.9%
36-B	+Nitrogen in the headspace	0.7%	0.6%	0.7%	1%	1.5%
37-B	+Air in the headspace + EDTA	0.7%	0.6%	0.8%	0.9%	1.5%
38-B	+Nitrogen in the headspace + EDTA	0.7%	0.6%	0.6%	0.8%	1.1%

[0514] As can be seen in Table 25, the EDTA containing composition, the nitrogen gas composition, and the EDTA plus nitrogen gas composition showed lower levels of aggregation over time as compared to a composition without EDTA and having air in the headspace.

Fragmentation Analysis:

[0515] The antibody compositions prepared according to Table 23 were stored at a temperature of 40° C. At weeks 0, 4, 8, 12 and 24, each composition was analyzed for total hydrolytic impurities (i.e., fragmentation) using reduced SDS-PAGE (rSDS-PAGE). The composition vials were aseptically sampled at each time point and loaded onto NuPAGE 4-12% bis-Tris gels with colloidal blue (Coomassie) stain. Gel reduction was achieved by use of the NuPAGE® reducing agent. The percentage impurity (i.e., fragmentation) of each sample band in the reduced gels was estimated by scanning on either a Molecular Dynamics Personal Densitometer PDQC-90 or Bio-Rad GS800 Imaging Densitometer. Fragmentation level was calculated as a percentage of total band volume (see Table 26).

TABLE 26

Percent Total (Impurities) Fragmentation for Compositions in Table 23:

Composition No.	Treatment	0 weeks Initial	4 weeks 40° C.	8 weeks 40° C.	12 weeks 40° C.	24 weeks 40° C.
35-B	+Air in the headspace	1.3%	4.1%	5.5%	8.0%	12.4%
36-B	+Nitrogen in the headspace	1.2%	3.5%	4.4%	6.3%	10.6%
37-B	+Air in the headspace + EDTA	1.3%	3.2%	4.4%	5.9%	10.6%
38-B	+Nitrogen in the headspace + EDTA	1.3%	3.5%	4.1%	5.6%	10.2%

[0516] As can be seen in Table 26, the EDTA containing composition, the nitrogen gas composition, and the EDTA plus nitrogen gas composition showed lower levels of fragmentation over time as compared to a composition without EDTA and having air in the headspace.

Formation of Acidic and Basic Species:

[0517] The antibody compositions prepared according to Table 23 were stored at a temperature of 40° C. At weeks 0, 4, 12 and 24, each composition was analyzed for the formation of acidic and basic species using Imaging Capillary Electrophoresis (ICE). The Imaging Capillary Electrophoresis was conducted using a Convergent Biosciences iCE_{280} analyzer for evaluation of charge heterogeneity. The Convergent iCE_{280} is an imaging capillary isoelectric focusing (IEF) instrument, which allows the user to take an image of a separated sample contained within a capillary.

[0518] The composition vials were aseptically sampled at each time point. The samples were then prepared in a mixture of electrophoretic ampholytes, methyl cellulose, calibration markers, and water. The samples were introduced into the iCE_{280} and a high potential/voltage was applied. The IEF assays were conducted using manually prepared pH 3-10.5 polyacrylamide gels using Coomassie blue stair. The sample protein components were separated based on their relative isoelectric points (pl) and their location. The relative amount of each separated component was observed by an imaging CCD camera. The data was then processed and reported as loss of the main peak (i.e., formation of acidic and basic species) using conventional chromatography integration software (see Table 27).

TABLE 27

Loss of Main Peak for Compositions in Table 23:

Composition No.	Treatment	0 weeks Initial	4 weeks 40° C.	8 weeks 40° C.	12 weeks 40° C.	24 weeks 40° C.
35-B	+Air in the headspace	65.3	50.3	—	28.8	15.5
36-B	+Nitrogen in the headspace	63.8	52.2	—	33.1	22.7

TABLE 27-continued

Loss of Main Peak for Compositions in Table 23:

Composition No.	Treatment	0 weeks Initial	4 weeks 40° C.	8 weeks 40° C.	12 weeks 40° C.	24 weeks 40° C.
37-B	+Air in the headspace + EDTA	62.3	55.4	—	37.0	23.4
38-B	+Nitrogen in the headspace + EDTA	63.9	56.6	—	40.0	24.8

[0519] As can be seen in Table 27, the EDTA containing composition, the nitrogen gas composition, and the EDTA plus nitrogen gas composition showed higher levels of the intact main peak over time as compared to a composition without EDTA and having air in the headspace. Thus, the amount of acidic and basic species formation is greater over time in compositions lacking EDTA and/or nitrogen gas in the headspace.

Amino Acid Oxidation Analysis:

[0520] Oxidation levels of methionine residues at amino acid positions 256 and 432 in anti-CTLA-4 antibody 11.2.1 were measured by a Lysine-C mapping method after storage for 12 weeks at 40° C.

[0521] The vials containing the compositions from Table 23 were aseptically sampled at the 12 week time point. The samples were then digested with a Lysyl Endopeptidase (Lys-C) enzyme tris buffer at pH 8.0 under standard conditions and analyzed by reversed-phase high performance liquid chromatography. Separation was accomplished using a Grace Vydac Protein C4 analytical column with 0.1% TFA in water and 0.085% TFA in Acetonitrile gradient elution.

TABLE 28

Percent Oxidation of Methionine Amino Acids in Anti-CTLA-4 antibody 11.2.1 in Compositions from Table 23:

Composition No.	Treatment	Amino Acid Residue	Percent Oxidation 0 weeks	Percent Oxidation 12 weeks
35-B	+Air in the headspace	Met-432	1.6%	7.3%
		Met-256	5%	17.9%
36-B	+Nitrogen in the headspace	Met-432	1.6%	3.3%
		Met-256	5%	7.8%
37-B	+Air in the headspace + EDTA	Met-432	1.6%	3.9%
		Met-256	5%	9.7%
38-B	+Nitrogen in the headspace + EDTA	Met-432	1.6%	2.6%
		Met-256	5%	5.9%

[0522] The results in Table 28 indicate that the addition of EDTA to the 11.2.1 antibody composition and/or addition of nitrogen gas to the vial headspace reduced the percent oxidation at the two indicated methionine residues as compared to a composition without EDTA and having air in the headspace.

EXAMPLE 10-B

[0523] A study was conducted to compare the effect on stability of anti-CTLA-4 antibody 11.2.1 compositions comprising a sodium acetate buffer and sodium chloride (i.e., chloride ions) versus compositions comprising a histidine buffer and trehalose.

[0524] Specifically, the impact on antibody 11.2.1 stability was analyzed with regards to discoloration, aggregation, and fragmentation.

[0525] The compositions that were evaluated are listed in Table 29 below. The procedure used to prepare the compositions is the same as the one described in Example 3-B.

[0526] The compositions in Table 29 were prepared by taking an 11.9 mg/ml stock solution of antibody 11.2.1 in 20 mM sodium acetate buffer pH 5.5, 140 mM sodium chloride and subjecting it to an ultrafiltration/diafiltration (UF/DF) step in a Millipore Lab Scale TFF System with a Pellicon XL PBTK 30K 50 cm membrane. Next, concentrated solutions of the antibody 11.2.1 were prepared in the 35 to 40 mg/ml range in 20 mM sodium acetate or 20 mM histidine buffers.

[0527] Concentrates of the tonicifying agent were prepared in either the sodium acetate or histidine buffer at three times the target final concentrations. A concentrated solution of polysorbate 80 was prepared at 20 mg/ml and $Na_2EDTA.2H_2O$ at 10 mg/ml in each of the buffers. Individual compositions were prepared by diluting the concentrated solutions appropriately. The compositions were then filtered through 0.2μ sterilizing grade filters and filled into several duplicate vials. A fill-volume of 1 ml was used in 2-ml Type 1 glass vials. The vials were closed with Dalkyo 777-1 Fluorotec® coated stoppers, crimp sealed, and stored upright in stability chambers at 25° C. and 40° C. Another set of vials was also placed at −20° C. for 4 weeks, and another set was subject to 4× freeze/thaw cycles (water-filled vials box) as described in Example 4-B. All compositions had a pH of 5.5 and an anti-CTLA-4 antibody 11.2.1 concentration of 20 mg/ml.

[0528] Several vials were immediately analyzed for levels of discoloration, aggregation, and fragmentation and several other duplicate vials were also stored upright at 25° C. and 40° C. for 4, 8, 12, 18, 24 and 36 weeks. At each time point, two stored vials per composition were removed from each condition to measure the level of antibody 11.2.1 aggregation, fragmentation, and observed for discoloration as well. Tables 30 through 34 and FIGS. **15** and **16** report the results.

TABLE 29

Antibody Compositions Tested:

No.	Acetate (mM)	Histidine (mM)	Tween 80 (mg/ml)	Sodium Chloride (mg/ml)	Mannitol (mg/ml)	Trehalose (mg/ml)	Glycine (mg/ml)	EDTA (mg/ml)	PEG 3350 (mg/ml)	Methionine (mg/ml)
26-B	20	—	0.2	8.2	—	—	—	—	—	—
39-B	20	—	0.2	5.0	18	—	—	0.1	—	—
40-B	—	20	0.2	—	—	84	—	—	—	—
37-B	—	20	0.2	—	—	84	—	0.1	—	—
41-B	—	20	0.2	—	—	84	—	0.1	—	2
42-B	—	20	0.4	—	—	84	—	0.1	—	—
43-B	—	20	0.2	—	41	10	—	—	—	—
44-B	—	20	0.2	—	41	10	—	0.1	—	—

Composition Appearance Analysis:

[0529] Each composition was visually evaluated after 1) initially, mixing the composition, 2) freezing the composition at −20° C. for 4 weeks, and 3) after 4 freeze/thaw cycles (−70° C. to 5° C. in box along with water-filled vials as described in Example 4-B). Each composition was visually evaluated after 0 (initial), 8, 12 and 24 weeks for particulate formation, color change and turbidity change. The compositions were evaluated for particulate formation, color changes and turbidity changes and reported in Table 30 (freeze/thaw), Table 31 (storage at 25° C.), and Table 32 (storage at 40° C.).

TABLE 30

Visual Evaluations of Compositions from Table 29 after Freeze/Thaw:

No.	Initial	After Freezing at −20° C. for 4 weeks	After 4 Freeze/Thaw Cycles
26-B	clear, colorless, no particulates	very slightly cloudy, more than 3 particulates	very slightly cloudy, many particulates
39-B	clear, colorless, no particulates	clear, colorless, no particulates	clear, colorless, less than 3 particulates
40-B	clear, colorless, no particulates	clear, colorless, no particulates	clear, colorless, less than 3 particulates
37-B	clear, colorless, no particulates	clear, colorless, no particulates	clear, colorless, less than 3 particulates
41-B	clear, colorless, no particulates	clear, colorless, no particulates	clear, colorless, less than 3 particulates
42-B	clear, colorless, no particulates	clear, colorless, no particulates	clear, colorless, less than 3 particulates
43-B	clear, colorless, no particulates	clear, colorless, no particulates	clear, colorless, less than 3 particulates
44-B	clear, colorless, no particulates	clear, colorless, less than 3 particulates	clear, colorless, no particulates

TABLE 31

Visual Evaluations of Compositions from Table 29 after Storage at 25° C.:

No.	Initial	8 weeks 25° C.	12 weeks 25° C.	24 weeks 25° C.
26-B	clear, colorless, no particulates	very slightly cloudy, no particulates	clear, colorless, no particulates	clear, colorless, less than 3 particulates
39-B	clear, colorless, no particulates	clear, colorless, no particulates	clear, colorless, less than 3 particulates	—

TABLE 31-continued

Visual Evaluations of Compositions from Table 29 after Storage at 25° C.:

No.	Initial	8 weeks 25° C.	12 weeks 25° C.	24 weeks 25° C.
40-B	clear, colorless, no particulates	clear, colorless, no particulates	clear, colorless, no particulates	clear, colorless, no particulates
37-B	clear, colorless, no particulates	clear, colorless, no particulates	clear, colorless, no particulates	clear, colorless, no particulates
41-B	clear, colorless, no particulates	clear, colorless, no particulates	clear, colorless, no particulates	clear, colorless, no particulates
42-B	clear, colorless, no particulates	clear, colorless, no particulates	clear, colorless, no particulates	clear, colorless, no particulates
43-B	clear, colorless, no particulates	—	clear, colorless, no particulates	clear, colorless, no particulates
44-B	clear, colorless, no particulates	clear, colorless, no particulates	clear, colorless, no particulates	very slightly cloudy, no particulates

TABLE 32

Visual Evaluations of Compositions from Table 29 after Storage at 40° C.:

No.	Initial	8 weeks 40° C.	12 weeks 40° C.	24 weeks 40° C.
26-B	clear, colorless, no particulates	very slightly cloudy, no particulates	clear, colorless, no particulates	slightly pink, no particulates
39-B	clear, colorless, no particulates	clear, colorless, no particulates	clear, colorless, no particulates	—
40-B	clear, colorless, no particulates	clear, colorless, no particulates	clear, colorless, no particulates	slightly pink, no particulates
37-B	clear, colorless, no particulates	clear, colorless, no particulates	clear, colorless, no particulates	clear, colorless, no particulates
41-B	clear, colorless, no particulates	clear, colorless, no particulates	clear, colorless, no particulates	clear, colorless, no particulates
42-B	clear, colorless, no particulates	clear, colorless, no particulates	clear, colorless, no particulates	clear, colorless, no particulates
43-B	clear, colorless, no particulates	clear, colorless, no particulates	clear, colorless, no particulates	slightly pink, no particulates
44-B	clear, colorless, no particulates	clear, colorless, no particulates	clear, colorless, no particulates	clear, colorless, no particulates

[0530] The results in Tables 20 through 22 indicate that antibody 11.2.1 compositions containing EDTA had reduced discoloration, reduced turbidity, and reduced particulate formation as compared to those compositions without EDTA. Overall, compositions containing sodium chloride had increased discoloration, turbidity, and particulate formation as compared to compositions having EDTA, but without sodium chloride.

Aggregation Analysis:

[0531] The antibody compositions prepared according to Table 29 were stored at a temperature of 25° C. and 40° C. At weeks 0 (initial), 4, 8, 12, 24 and 36 weeks, the 25° C. compositions were analyzed for aggregation using size exclusion chromatography. At weeks 4, 8, 12 and 24 weeks, the 40° C. compositions were analyzed for aggregation using size exclusion chromatography. The composition vials were aseptically sampled at each time point. The size exclusion chromatography was carried out on the samples using a TSK gel G3000SWXL-G2000SWXL column, mobile phase 0.2 M sodium phosphate buffer at pH 7.0, a flow rate of 1 ml/min, and UV detection at 214 nm. Tables 33(a) and 33(b) show the percentage of antibody 11.2.1 aggregation measured at the relevant times for each of the composition treatments. Aggregation levels were calculated by integrating the areas under the chromatogram peaks for each composition and reporting the integrated areas under the high molecular weight species peaks as a percentage of total peak area (see Tables 33(a) and 33(b)).

TABLE 33(a)

Percent Aggregation for Compositions in Table 29 after Storage at 25° C.:

No.	Initial	4 weeks 25° C.	8 weeks 25° C.	12 weeks 25° C.	18 weeks 25° C.	24 weeks 25° C.	36 weeks 25° C.
26-B	0.7%	0.9%	0.9%	1.1%	1.8%	1.8%	3.0%
39-B	0.7%	0.8%	0.8%	1.1%	—	—	—
40-B	0.6%	0.6%	0.7%	0.9%	0.95%	0.8%	0.9%
37-B	0.6%	0.6%	0.6%	0.7%	0.7%	0.7%	0.8%
41-B	0.6%	0.6%	0.6%	0.7%	0.7%	0.7%	0.7%
42-B	0.6%	0.6%	0.6%	0.8%	0.8%	0.8%	0.8%
43-B	0.6%	0.6%	—	—	0.7%	0.8%	0.9%
44-B	0.6%	0.6%	0.6%	0.7%	0.7%	0.8%	0.8%

[0532] Table 33(b) below reports the aggregation data that is graphically presented in FIG. **15**.

TABLE 33(b)

Percent Aggregation for Compositions in Table 29 after Storage at 40° C.:

No.	Initial	4 weeks 40° C.	8 weeks 40° C.	12 weeks 40° C.	24 weeks 40° C.
26-B	0.7%	1.0%	1.3%	2.8%	4.7%
39-B	0.6%	1.0%	1.0%	1.4%	—
40-B	0.6%	0.7%	0.9%	1.0%	1.4%
37-B	0.6%	0.6%	0.6%	0.8%	1.1%
41-B	0.6%	0.6%	0.6%	0.7%	0.8%
42-B	0.6%	0.6%	0.6%	0.8%	1.1%
43-B	0.6%	0.8%	—	—	1.7%
44-B	0.6%	0.6%	0.6%	0.8%	0.9%

[0533] As can be seen in Tables 33(a), 33(b) and FIG. **15**, the EDTA-containing compositions showed reduced levels of aggregation as compared to a composition lacking EDTA, but having an acetate buffer and sodium chloride, after storage at 25° C. and 40° C. Moreover, a composition containing a histidine buffer (without EDTA) had a reduced amount of aggregation compared to a composition lacking EDTA, but containing an acetate buffer and sodium chloride.

Fragmentation Analysis:

[0534] The antibody compositions prepared according to Table 29 were stored at a temperature of 25° C. and 40° C. At weeks 0 (initial), 4, 8, 12, 18 and 36 weeks, each composition was analyzed for total hydrolytic impurities (i.e., fragmentation) using reduced SDS-PAGE (rSDS-PAGE). The composition vials were aseptically sampled at each time point and loaded onto NuPAGE 4-12% bis-Tris gels with colloidal blue (Coomassie) stain. Gel reduction was achieved by use of the NuPAGE® reducing agent. The percentage impurity (i.e., fragmentation) of each sample band in the reduced gels was estimated by scanning on either a Molecular Dynamics Personal Densitometer PDQC-90 or Bio-Rad GS800 Imaging Densitometer. Fragmentation level was calculated as a percentage of total band volume (see Tables 34(a) and 34(b)).

TABLE 34(a)

	Percent Fragmentation for Compositions in Table 29 after Storage at 25° C.:						
No.	Initial	4 weeks 25° C.	8 weeks 25° C.	12 weeks 25° C.	18 weeks 25° C.	24 weeks 25° C.	36 weeks 25° C.
26-B	1.8%	1.6%	2.6%	2.4%	1.3%	3.5%	3.7%
39-B	1.6%	1.4%	2.4%	1.5%	—	—	—
40-B	1.6%	1.7%	2.6%	1.4%	1.4%	3.3%	3.2%
37-B	1.6%	1.6%	2.5%	1.4%	1.4%	3.3%	3.1%
41-B	1.6%	1.6%	2.6%	1.4%	1.2%	3.3%	2.9%
42-B	1.9%	1.8%	2.5%	1.6%	1.2%	3.0%	2.9%
43-B	1.8%	1.8%	—	—	1.2%	3.2%	3.1%
44-B	1.7%	1.7%	2.6%	1.4%	1.3%	3.1%	2.8%

[0535] Table 34(b) below reports the fragmentation data that is graphically presented in FIG. **16**.

TABLE 34(b)

	Percent Fragmentation for Compositions in Table 29 after Storage at 40° C.:				
No.	Initial	4 weeks 40° C.	8 weeks 40° C.	12 weeks 40° C.	24 weeks 40° C.
26-B	1.8%	5.2%	6.1%	7.8%	11.7%
39-B	1.6%	5.3%	6.7%	6.5%	—
40-B	1.6%	5.3%	6.8%	6.2%	10.0%
37-B	1.6%	5.2%	6.6%	5.5%	10.2%
41-B	1.6%	5.2%	5.0%	5.5%	10.0%
42-B	1.9%	5.3%	5.1%	5.8%	9.7%
43-B	1.8%	5.3%	—	—	11.0%
44-B	1.7%	4.5%	5.2%	5.5%	9.5%

[0536] As can be seen in Tables 34(a), 34(b) and FIG. **16**, the EDTA-containing compositions showed reduced levels of fragmentation as compared to a composition lacking EDTA, but having an acetate buffer and sodium chloride, after storage at 25° C. and 40° C.

EXAMPLE 11-B

[0537] A study was conducted to compare the effect of varying concentrations of EDTA on stability of anti-CTLA-4 antibody 11.2.1 compositions. Alternatives to a histidine buffer-trehalose composition were also tested, by replacing part of the trehalose with mannitol.

[0538] Specifically, the impact on antibody 11.2.1 stability was analyzed with regards to discoloration, aggregation, fragmentation, and oxidation.

[0539] The compositions that were evaluated are listed in Table 35 below. The procedure used to prepare the compositions is the same as the one described in Example 10-B.

[0540] The compositions in Table 35 were prepared by taking an 11.9 mg/ml stock solution of antibody 11.2.1 in 20 mM sodium acetate buffer pH 5.5, 140 mM sodium chloride and subjecting it to an ultrafiltration/diafiltration (UF/DF) step in a Millipore Lab Scale TFF System with a Pellicon XL PBTK 30K 50 cm^2 membrane. Next, concentrated solutions of the antibody 11.2.1 were prepared in the 35 to 40 mg/ml range in 20 mM sodium acetate or 20 mM histidine buffers.

[0541] Concentrates of the tonicifying agent were prepared in either the sodium acetate or histidine buffer at three times the target final concentrations. A concentrated solution of polysorbate 80 was prepared at 20 mg/ml and $Na_2EDTA.2H_2O$ at 10 mg/ml in each of the buffers. Individual compositions were prepared by diluting the concentrated solutions appropriately. EDTA concentrations (as $Na_2EDTA.2H_2O$) were examined in the range of 0-0.1 mg/ml. The compositions were then filtered through 0.2μ sterilizing grade filters and filled into several duplicate vials. A fill-volume of 1 ml was used in 2-ml Type 1 glass vials.

[0542] The vials were closed with Dalkyo 777-1 Fluorotec® coated stoppers, crimp sealed, and stored upright in stability chambers at 25° C. and 40° C. Another set of vials was subject to 4× freeze/thaw cycles as described in Example 10-B. All compositions had a pH of 5.5 and an anti-CTLA-4 antibody 11.2.1 concentration of 20 mg/ml.

[0543] Several vials were immediately analyzed for levels of discoloration, aggregation, fragmentation, and oxidation and several other duplicate vials were also stored upright at 25° C. and 40° C. for 4, 8, 13, 18 and 24 weeks. At each time point, two stored vials per composition from each condition were removed to measure the level of antibody 11.2.1 aggregation, fragmentation, and observed for discoloration as well. Tables 36 to 41 and FIGS. **17** to **22** report the results.

TABLE 35

	Antibody Compositions Tested:						
No.	Acetate (mM)	Histidine (mM)	Tween 80 (mg/ml)	Mannitol (mg/ml)	Trehalose (mg/ml)	EDTA (mg/ml)	Sodium Chloride (mg/ml)
26-B	20	—	0.2	—	—	—	8.4
40-B	—	20	0.2	—	84	—	—
45-B	—	20	0.2	—	84	0.001	—
46-B	—	20	0.2	—	84	0.005	—
47-B	—	20	0.2	—	84	0.01	—
48-B	—	20	0.2	—	84	0.05	—
37-B	—	20	0.2	—	84	0.1	—
49-B	—	20	0.2	10	70	0.001	—
50-B	—	20	0.4	10	70	0.01	—
51-B	—	20	0.2	10	70	0.1	—
52-B	—	20	0.2	20	50	0.001	—
53-B	—	20	0.2	20	50	0.01	—
54-B	—	20	0.2	20	50	0.1	—

Composition Appearance Analysis:

[0544] Each composition was visually evaluated after 1) initially mixing the composition, 2) after 4 freeze/thaw cycles, and 3) after storage at 25° C. and 40° C. for 4, 8, 13, 18 and 24 weeks. The compositions were evaluated for particulate formation, color changes and turbidity changes and reported in Tables 36 to 38.

TABLE 36

Visual Evaluations of Compositions from Table 35 after Freeze/Thawing:

Composition No.	Initial	After 4X Freeze/Thaw Cycles
26-B	clear, colorless, no particulates	very slightly cloudy, no particulates
40-B	clear, colorless, no particulates	clear, colorless, less than 3 particulates
45-B	clear, colorless, no particulates	clear, colorless, less than 3 particulates
46-B	clear, colorless, no particulates	clear, colorless, less than 3 particulates
47-B	clear, colorless, no particulates	clear, colorless, less than 3 particulates
48-B	clear, colorless, no particulates	clear, colorless, greater than 3 particulates
37-B	clear, colorless, no particulates	clear, colorless, no particulates
49-B	clear, colorless, no particulates	clear, colorless, greater than 3 particulates
50-B	clear, colorless, no particulates	clear, colorless, less than 3 particulates
51-B	clear, colorless, no particulates	clear, colorless, less than 3 particulates
52-B	clear, colorless, no particulates	clear, colorless, no particulates
53-B	clear, colorless, no particulates	clear, colorless, less than 3 particulates
54-B	clear, colorless, no particulates	clear, colorless, less than 3 particulates

TABLE 37

Visual Evaluations of Compositions from Table 35 after Storage at 25° C.:

No.	Initial	13 weeks 25° C.	18 weeks 25° C.	24 weeks 25° C.
26-B	clear, colorless, no particulates	clear, colorless, no particulates	very slightly pink, no particulates	
40-B	clear, colorless, no particulates	very slightly pink, no particulates	—	clear, Y6*, no particulates
45-B	clear, colorless, no particulates	clear, colorless, no particulates	clear, colorless, no particulates	clear, colorless, no particulates
46-B	clear, colorless, no particulates	clear, colorless, no particulates	—	clear, colorless, no particulates
47-B	clear, colorless, no particulates	clear, colorless, no particulates	clear, colorless, no particulates	clear, colorless, no particulates
48-B	clear, colorless, no particulates	clear, colorless, no particulates	—	clear, colorless, no particulates
37-B	clear, colorless, no particulates	clear, colorless, no particulates	clear, colorless, no particulates	clear, colorless, no particulates
49-B	clear, colorless, no particulates	clear, colorless, no particulates	—	clear, colorless, no particulates
50-B	clear, colorless, no particulates	clear, colorless, less than 3 particulates	—	clear, colorless, no particulates
51-B	clear, colorless, no particulates	clear, colorless, less than 3 particulates	—	clear, colorless, no particulates
52-B	clear, colorless, no particulates	clear, colorless, no particulates	—	clear, colorless, no particulates
53-B	clear, colorless, no particulates	clear, colorless, no particulates	—	clear, colorless, no particulates
54-B	clear, colorless, no particulates	clear, colorless, less than 3 particulates	—	clear, colorless, no particulates

*Y6 and Y4 are color scale notations on the EP Yellow scale. Y6 being less yellow than Y4. (Ref: PhEur 5.0, 2005 Monograph 2.2.2).

TABLE 38

Visual Evaluations of Compositions from Table 35 after Storage at 40° C.:

No.	Initial	8 weeks 40° C.	13 weeks 40° C.	18 weeks 40° C.	24 weeks 40° C.
26-B	clear, colorless, no particulates	very slightly pink, no particulates	very slightly pink, no particulates	pink, no particulates	pink, no particulates
40-B	clear, colorless, no particulates	very slightly pink, no particulates	very slightly pink, no particulates	—	clear, Y4*, no particulates
45-B	clear, colorless, no particulates	clear, colorless, no particulates	clear, colorless, no particulates	clear, colorless, no particulates	clear, colorless, no particulates
46-B	clear, colorless, no particulates	clear, colorless, no particulates	clear, colorless, no particulates	—	clear, colorless, no particulates
47-B	clear, colorless, no particulates	clear, colorless, no particulates	clear, colorless, less than 3 particulates	clear, colorless, no particulates	clear, colorless, no particulates
48-B	clear, colorless, no particulates	clear, colorless, no particulates	clear, colorless, no particulates	—	clear, colorless, no particulates
37-B	clear, colorless, no particulates	clear, colorless, no particulates	clear, colorless, no particulates	clear, colorless, no particulates	clear, colorless, no particulates
49-B	clear, colorless, no particulates	clear, colorless, less than 3 particulates	clear, colorless, no particulates	—	clear, colorless, no particulates
50-B	clear, colorless, no particulates	clear, colorless, less than 3 particulates	clear, colorless, less than 3 particulates	—	clear, colorless, no particulates
51-B	clear, colorless, no particulates	clear, colorless, no particulates	clear, colorless, no particulates	—	clear, colorless, no particulates

TABLE 38-continued

Visual Evaluations of Compositions from Table 35 after Storage at 40° C.:

No.	Initial	8 weeks 40° C.	13 weeks 40° C.	18 weeks 40° C.	24 weeks 40° C.
52-B	clear, colorless, no particulates	clear, colorless, no particulates	clear, colorless, less than 3 particulates	—	clear, colorless, no particulates
53-B	clear, colorless, no particulates	clear, colorless, no particulates	clear, colorless, no particulates	—	clear, colorless, no particulates
54-B	clear, colorless, no particulates	clear, colorless, no particulates	clear, colorless, less than 3 particulates	—	clear, colorless, no particulates

*Y6 and Y4 are color scale notations on the EP Yellow scale. Y6 being less yellow than Y4. (Ref: PhEur 5.0, 2005 Monograph 2.2.2).

[0545] The results in Tables 36 through 38 indicate that antibody 11.2.1 compositions containing all tested EDTA concentrations had reduced discoloration, reduced turbidity, and reduced particulate formation as compared to those compositions without EDTA.

[0546] Overall, compositions containing sodium chloride had reduced freeze/thaw protection as evidenced by increased discoloration, turbidity, and particulate formation as compared to compositions having EDTA, but without sodium chloride.

Aggregation Analysis:

[0547] The antibody compositions prepared according to Table 35 were stored at a temperature of 25° C. and 40° C. At weeks, 0 (initial), 4, 8, 13, 18 and 24, the 25° C. and 40° C. compositions were analyzed for aggregation using size exclusion chromatography. The composition vials were aseptically sampled at each time point. The size exclusion chromatography was carried out on the samples using a TSK gel G3000SWXL-G2000SWXL column, mobile phase 0.2 M sodium phosphate buffer at pH 7.0, a flow rate of 1 ml/min, and UV detection at 214 nm. Table 39(a) shows the percentage of antibody 11.2.1 aggregation measured after storage at 25° C. at the relevant times for each of the compositions. Table 39(b) shows the percentage of antibody 11.2.1 aggregation measured after storage at 40° C. Aggregation levels were calculated by integrating the areas under the chromatogram peaks for each composition and reporting the integrated areas under the high molecular weight species peaks as a percentage of total peak area (see Tables 39(a) and 39(b)).

TABLE 39(a)

Percent Aggregation for Compositions in Table 25 after Storage at 25° C.:

No.	Initial	4 weeks 25° C.	8 weeks 25° C.	13 weeks 25° C.	18 weeks 25° C.	24 weeks 25° C.
26-B	0.8%	—	—	1.1%	1.6%	—
40-B	0.7%	—	—	0.7%	0.8%	0.8%
45-B	0.7%	0.6%	0.6%	0.6%	0.7%	0.7%
46-B	0.7%	—	—	0.6%	0.7%	0.7%
47-B	0.7%	0.7%	0.6%	0.6%	0.7%	0.7%
48-B	0.7%			0.6%	0.7%	0.7%
37-B	0.7%	0.6%	0.6%	0.6%	0.7%	0.7%
49-B	0.7%	—	—	0.6%	—	—
50-B	0.7%	—	—	0.6%	—	—
51-B	0.7%	—	—	0.6%	—	0.7%

TABLE 39(a)-continued

Percent Aggregation for Compositions in Table 25 after Storage at 25° C.:

No.	Initial	4 weeks 25° C.	8 weeks 25° C.	13 weeks 25° C.	18 weeks 25° C.	24 weeks 25° C.
52-B	0.7%	0.6%	0.6%	0.6%	—	—
53-B	0.7%	0.6%	0.6%	0.6%	—	0.7%
54-B	0.7%	0.6%	0.6%	0.6%	—	—

[0548] Table 39(b) below reports the aggregation data that is graphically presented in FIG. **17**.

TABLE 39(b)

Percent Aggregation for Compositions in Table 25 after Storage at 40° C.:

No.	Initial	4 weeks 40° C.	8 weeks 40° C.	13 weeks 40° C.	18 weeks 40° C.	24 weeks 40° C.
26-B	0.8%	—	3.1%	4.3%	5.2%	—
40-B	0.7%	—	0.9%	1.2%	1.8%	2.7%
45-B	0.7%	0.6%	0.8%	0.8%	1.0%	1.4%
46-B	0.7%	—	0.7%	0.8%	1.0%	1.3%
47-B	0.7%	0.6%	0.7%	0.8%	0.8%	1.1%
48-B	0.7%	—	0.7%	0.8%	0.9%	1.3%
37-B	0.7%	0.7%	0.7%	0.8%	1.0%	1.1%
49-B	0.7%	—	0.8%	0.8%	—	—
50-B	0.7%	—	0.7%	0.8%	—	—
51-B	0.7%	—	0.8%	0.8%	—	1.2%
52-B	0.7%	0.6%	0.7%	0.8%	—	—
53-B	0.7%	0.7%	0.7%	0.7%	—	1.2%
54-B	0.7%	0.6%	0.8%	0.7%	—	—

[0549] As can be seen in Tables 39(a), 39(b) and FIG. **17**, the EDTA-containing compositions showed reduced levels of aggregation at all tested EDTA concentrations as compared to a composition lacking EDTA, but having an acetate buffer and sodium chloride, after storage at 25° C. and 40° C. FIG. **17** graphically summarizes the reduction in percent aggregation for the compositions from Table 35 as a function of EDTA concentration.

Fragmentation Analysis:

[0550] The antibody compositions prepared according to Table 35 were stored at a temperature of 25° C. and 40° C. At weeks, 0 (initial), 4, 8, 13, 18 and 24, the 25° C. and 40° C. compositions were analyzed for total hydrolytic impurities

(i.e., fragmentation) using reduced SDS-PAGE (rSDS-PAGE). The composition vials were aseptically sampled at each time point and loaded onto NuPAGE 4-12% bis-Tris gels with colloidal blue (Coomassie) stain. Gel reduction was achieved by use of the NuPAGE® reducing agent. The percentage impurity (i.e., fragmentation) of each sample band in the reduced gels was estimated by scanning on either a Molecular Dynamics Personal Densitometer PDQC-90 or Bio-Rad GS800 Imaging Densitometer. Fragmentation level was calculated as a percentage of total band volume (see Tables 40(a) and 40(b)).

TABLE 40(a)

	Percent Fragmentation for Compositions in Table 35 after Storage at 25° C.:					
No.	Initial	4 weeks 25° C.	8 weeks 25° C.	13 weeks 25° C.	18 weeks 25° C.	24 weeks 25° C.
26-B	1.3%	—	—	3.3%	3.7%	—
40-B	1.1%	—	—	2.6%	3.1%	3.1%
45-B	1.5%	2.7%	2.3%	3.1%	3.0%	3.4%
46-B	1.0%	—		2.5%	3.1%	2.9%
47-B	2.5%	2.5%	2.3%	3.1%	3.0%	3.3%
48-B	3.1%	—	—	2.5%	3.2%	2.9%
37-B	3.6%	2.6%	2.3%	3.1%	3.0%	3.3%
49-B	1.0%	—	—	2.6%	—	—
50-B	1.1%	—	—	2.7%	—	—
51-B	1.2%	—	—	2.7%	—	2.9%
52-B	2.4%	2.7%	2.3%	3.1%	—	—
53-B	1.6%	2.4%	2.3%	2.9%	—	3.4%
54-B	1.6%	2.7%	2.2%	3.2%	—	—

[0551] Table 40(b) below reports the fragmentation data that is graphically presented in FIG. **18**.

TABLE 40(b)

	Percent Fragmentation for Compositions in Table 35 after Storage at 40° C.:					
No.	Initial	4 weeks 40° C.	8 weeks 40° C.	13 weeks 40° C.	18 weeks 40° C.	24 weeks 40° C.
26-B	—	—	6.2%	7.3%	8.7%	10.1%
40-B	—	—	3.9%	6.9%	8.2%	7.2%
45-B	—	2.9%	4.3%	4.2%	6.7%	6.7%
46-B	—	—	4.1%	5.6%	7.1%	6.1%
47-B	—	1.9%	2.8%	3.4%	5.4%	5.6%
48-B	—	—	2.0%	4.2%	5.0%	4.0%
37-B	—	0.7%	1.5%	2.2%	5.0%	4.5%
49-B	—	—	4.1%	5.8%	—	—
50-B	—	—	3.7%	5.5%	—	—
51-B	—	—	3.7%	5.5%	—	5.4%
52-B	—	1.8%	2.4%	3.1%	—	—
53-B	—	2.4%	3.3%	4.1%	—	6.6%
54-B	—	2.3%	3.1%	3.8%	—	—

[0552] As can be seen in Tables 40(a), 40(b) and FIG. **18**, the EDTA-containing compositions showed reduced levels of fragmentation as compared to a composition lacking EDTA, but having an acetate buffer and sodium chloride, after storage at 25° C. and 40° C. In addition, the compositions containing histidine and trehalose without EDTA showed reduced fragmentation over the composition containing sodium chloride without EDTA.

[0553] FIG. **20** graphically summarizes the reduction in percent fragmentation for the compositions from Table 35 as a function of EDTA concentration.

Amino Acid Oxidation Analysis:

[0554] Oxidation levels of certain methionine residues at amino acid positions 256 and 432 in the anti-CTLA-4 antibody 11.2.1 were measured by a Lysine-C mapping method. The vials containing the compositions from Table 35 were aseptically sampled at the 18 week and 24 week time points after storage at 40° C. The samples were then digested with a Lysyl Endopeptidase (Lys-C) enzyme in tris buffer at pH 8.0 under standard conditions and analyzed by reversed-phase high performance liquid chromatography. Separation was accomplished using a Grace Vydac Protein C4 analytical column with 0.1% TFA in water and 0.085% TFA in Acetonitrile gradient elution. Table 41 reports the results.

TABLE 41

	Percent Oxidation of Methionine Amino Acids in Anti-CTLA-4 antibody 11.2.1 in Compositions from Table 35:			
No.	Amino Acid Residue	Initial	18 weeks	24 weeks
26-B	Met 432	1.6%	5.5%	6.9%
	Met 256	5%	12.8%	13.6%
40-B	Met 432	1.6%	5.6%	8.8%
	Met 256	5%	14.0%	16.1%
45-B	Met 432	1.6%	4.8%	6.2%
	Met 256	5%	11.6%	12.8%
46-B	Met 432	1.6%	3.5%	6.1%
	Met 256	5%	8.8%	12.5%
47-B	Met 432	1.6%	3.4%	4.2%
	Met 256	5%	8.4%	8.3%
48-B	Met 432	1.6%	2.8%	5.7%
	Met 256	5%	7.6%	12.6%
37-B	Met 432	1.6%	4.5%	4.7%
	Met 256	5%	11.0%	9.4%

[0555] As can be seen in Table 41, the presence of EDTA in the antibody 11.2.1 composition reduces the level of methionine oxidation that occurs over time.

EXAMPLE 12-B

[0556] A study was conducted to compare the effect mannitol and sorbitol on stability of anti-CTLA-4 antibody 11.2.1 compositions. In this Example, alternatives to a histidine-trehalose composition were tested, by replacing part of the trehalose with varying concentrations of mannitol and/or sorbitol (Table 32). EDTA concentrations (as $Na_2EDTA.2H_2O$) were examined in the range of 0 to 0.1 mg/ml.

[0557] Specifically, the impact on antibody 11.2.1 stability was analyzed with regards to discoloration, aggregation, fragmentation, and oxidation.

[0558] The compositions that were evaluated are listed in Table 42 below. The procedure used to prepare the compositions is the same as the one described in Example 10-B.

[0559] The compositions in Table 42 were prepared by taking an 11.9 mg/ml stock solution of antibody 11.2.1 in 20 mM sodium acetate buffer pH 5.5, 140 mM sodium chloride and subjecting it to an ultrafiltration/diafiltration (UF/DF) step in a Millipore Lab Scale TFF System with a Pellicon XL PBTK 30K 50 cm^2 membrane. Next, concentrated solutions of the antibody 11.2.1 were prepared in the 35 to 40 mg/ml range in 20 mM sodium acetate or 20 mM histidine buffers.

[0560] Concentrates of the tonicifying agent were prepared in either the sodium acetate or histidine buffer at three times the target final concentrations. A concentrated solution of polysorbate 80 was prepared at 20 mg/ml and $Na_2EDTA.2H_2O$ at 10 mg/ml in each of the buffers. Individual compositions were prepared by diluting the concentrated solutions appropriately. The compositions were then filtered through 0.2 g sterilizing grade filters and filled into several duplicate vials. A fill-volume of 1 ml was used in 2 ml Type 1 glass vials.

[0561] The vials were closed with Dalkyo 777-1 Fluorotec® coated stoppers, crimp sealed, and stored upright in stability chambers at 25° C. and 40° C. Another set of vials was subject to 4× freeze/thaw cycles as described in Example 10-B. All compositions had a pH of 5.5 and an anti-CTLA-4 antibody 11.2.1 concentration of 20 mg/ml.

[0562] Several vials were immediately analyzed for levels of discoloration, aggregation, fragmentation, and oxidation and several other duplicate vials were also stored upright at 25° C. and 40° C. for 4, 8, 13, 18 and 24 weeks. At each time point, two stored vials per composition were removed from each condition to measure the level of antibody 11.2.1 aggregation, fragmentation, and observed for discoloration as well. Tables 43 to 47 and FIGS. **21** and **22** report the results.

TABLE 42

Antibody Compositions Tested:

No.	Sodium Acetate (mM)	Histidine (mM)	PS80 mg/ml	Mannitol (mg/ml)	Sorbitol (mg/ml)	EDTA (mg/ml)	Sodium Chloride (mg/ml)
26-B	20	—	0.2	—	—	—	8.4
55-B	—	20	0.2	—	45	0.001	—
56-B	—	20	0.2	—	45	0.01	—
57-B	—	20	0.2	—	45	0.1	—
58-B	—	20	0.2	5	40	0.001	—
59-B	—	20	0.2	5	40	0.01	—
60-B	—	20	0.2	5	40	0.1	—
61-B	—	20	0.2	15	30	0.001	—
62-B	—	20	0.2	15	30	0.01	—
63-B	—	20	0.2	15	30	0.1	—

Composition Appearance Analysis:

[0563] Each composition was visually evaluated after 1) initially mixing the composition, 2) after 4 freeze/thaw cycles (−70° C. to 5° C. along with water-filled vials in box from Example 4) and 3) after storage at 25° C. and 40° C. for 8, 13, and 24 weeks. The compositions were evaluated for particulate formation, color changes and turbidity changes and reported in Tables 43 to 45.

TABLE 43

Visual Evaluations of Compositions from Table 42 after Freeze/Thawing:

No.	Initial	After 4X Freeze/Thaw Cycles
26-B	clear, colorless, no particulates	very slightly cloudy, no particulates
55-B	clear, colorless, no particulates	clear, colorless, less than 3 particulates
56-B	clear, colorless, no particulates	clear, colorless, less than 3 particulates
57-B	clear, colorless, no particulates	clear, colorless, no particulates
58-B	clear, colorless, no particulates	clear, colorless, less than 3 particulates
59-B	clear, colorless, no particulates	clear, colorless, less than 3 particulates
60-B	clear, colorless, no particulates	clear, colorless, less than 3 particulates
61-B	clear, colorless, no particulates	clear, colorless, no particulates
62-B	clear, colorless, no particulates	clear, colorless, no particulates
63-B	clear, colorless, no particulates	clear, colorless, no particulates

TABLE 44

Visual Evaluations of Compositions from Table 42 after Storage at 25° C.:

No.	Initial	13 weeks 25° C.	24 weeks 25° C.
26-B	clear, colorless, no particulates	clear, colorless, no particulates	—
55-B	clear, colorless, no particulates	clear, colorless, less than 3 particulates	clear, colorless, no particulates
56-B	clear, colorless, no particulates	clear, colorless, less than 3 particulates	clear, colorless, no particulates
57-B	clear, colorless, no particulates	clear, colorless, less than 3 particulates	clear, colorless, no particulates

TABLE 44-continued

Visual Evaluations of Compositions from Table 42 after Storage at 25° C.:

No.	Initial	13 weeks 25° C.	24 weeks 25° C.
58-B	clear, colorless, no particulates	clear, colorless, no particulates	clear, colorless, no particulates
59-B	clear, colorless, no particulates	clear, colorless, no particulates	clear, colorless, no particulates
60-B	clear, colorless, no particulates	clear, colorless, no particulates	clear, colorless, no particulates
61-B	clear, colorless, no particulates	clear, colorless, no particulates	clear, colorless, no particulates
62-B	clear, colorless, no particulates	clear, colorless, no particulates	clear, colorless, no particulates
63-B	clear, colorless, no particulates	clear, colorless, no particulates	clear, colorless, no particulates

TABLE 45

Visual Evaluations of Compositions from Table 42 after Storage at 40° C.:

No.	Initial	8 weeks 40° C.	13 weeks 40° C.	24 weeks 40° C.
26-B	clear, colorless, no particulates	very slightly pink, no particulates	very slightly pink, no particulates	pink, no particulates
55-B	clear, colorless, no particulates	clear, colorless, no particulates	clear, colorless, no particulates	clear, colorless, no particulates
56-B	clear, colorless, no particulates	clear, colorless, no particulates	clear, colorless, no particulates	clear, colorless, no particulates

TABLE 45-continued

Visual Evaluations of Compositions from Table 42 after Storage at 40° C.:

No.	Initial	8 weeks 40° C.	13 weeks 40° C.	24 weeks 40° C.
57-B	clear, colorless, no particulates	clear, colorless, less than 3 particulates	clear, colorless, less than 3 particulates	clear, colorless, no particulates
58-B	clear, colorless, no particulates	clear, colorless, no particulates	clear, colorless, less than 3 particulates	clear, colorless, no particulates
59-B	clear, colorless, no particulates	clear, colorless, less than 3 particulates	clear, colorless, no particulates	clear, colorless, no particulates
60-B	clear, colorless, no particulates	clear, colorless, less than 3 particulates	clear, colorless, no particulates	clear, colorless, no particulates
61-B	clear, colorless, no particulates	clear, colorless, no particulates	clear, colorless, less than 3 particulates	clear, colorless, no particulates
62-B	clear, colorless, no particulates	clear, colorless, less than 3 particulates	clear, colorless, no particulates	clear, colorless, no particulates
63-B	clear, colorless, no particulates	clear, colorless, no particulates	clear, colorless, no particulates	clear, colorless, no particulates

[0564] The results in Tables 43 through 45 indicate that antibody 11.2.1 compositions containing sodium chloride, but without EDTA, had reduced freeze/thaw protection as evidenced by increased discoloration, turbidity, and particulate formation as compared to compositions having EDTA, but without sodium chloride. The results also indicate that antibody 11.2.1 compositions containing all tested EDTA concentrations had reduced discoloration, reduced turbidity, and reduced particulate formation as compared to compositions without EDTA.

Aggregation Analysis:

[0565] The antibody compositions prepared according to Table 42 were stored at a temperature of 25° C. and 40° C. At weeks, 0 (initial), 4, 8, 13, 18 and 24, the 25° C. and 40° C. compositions were analyzed for aggregation using size exclusion chromatography. The composition vials were aseptically sampled at each time point. The size exclusion chromatography was carried out on the samples using a TSK gel G3000SWXL-G2000SWXL column, mobile phase 0.2 M sodium phosphate buffer at pH 7.0, a flow rate of 1 ml/min, and UV detection at 214 nm. Table 46(a) shows the percentage of antibody 11.2.1 aggregation measured after storage at 25° C. at the relevant times for each of the compositions. Table 46(b) shows the percentage of antibody 11.2.1 aggregation measured after storage at 40° C. Aggregation levels were calculated by integrating the areas under the chromatogram peaks for each composition and reporting the integrated areas under the high molecular weight species peaks as a percentage of total peak area (see Tables 46(a) and 46(b)).

TABLE 46(a)

Percent Aggregation for Compositions in Table 42 after Storage at 25° C.:

No.	Initial	4 weeks 25° C.	8 weeks 25° C.	13 weeks 25° C.	18 weeks 25° C.	24 weeks 25° C.
26-B	0.8%	—	—	1.1%	1.6%	—
55-B	0.7%	0.6%	0.6%	0.6%	—	—

TABLE 46(a)-continued

Percent Aggregation for Compositions in Table 42 after Storage at 25° C.:

No.	Initial	4 weeks 25° C.	8 weeks 25° C.	13 weeks 25° C.	18 weeks 25° C.	24 weeks 25° C.
56-B	0.7%	0.6%	0.7%	0.6%	—	—
57-B	0.7%	0.6%	0.6%	0.6%	—	0.7%
58-B	0.7%	—	—	0.6%	—	—
59-B	0.7%	—	—	0.6%	—	—
60-B	0.7%	—	—	0.6%	—	—
61-B	0.7%	0.7%	0.6%	0.6%	—	—
62-B	0.7%	0.6%	0.6%	0.6%	—	—
63-B	0.7%	0.6%	0.8%	0.6%	—	—

[0566] Table 46(b) below reports the aggregation data that is graphically presented in FIG. **21**.

TABLE 46(b)

Percent Aggregation for Compositions in Table 42 after Storage at 40° C.:

No.	Initial	4 weeks 40° C.	8 weeks 40° C.	13 weeks 40° C.	18 weeks 40° C.	24 weeks 40° C.
26-B	0.8%	3.1%	4.3%	5.2%	6.4%	—
55-B	0.6%	0.7%	0.8%	0.8%	—	—
56-B	0.6%	0.7%	0.8%	0.7%	—	—
57-B	0.7%	0.7%	0.8%	0.8%	—	1.2%
58-B	0.7%	—	0.8%	0.8%	—	—
59-B	0.7%	—	0.8%	0.8%	—	—
60-B	0.6%	—	0.7%	0.8%	—	—
61-B	0.7%	0.7%	0.7%	0.8%	—	—
62-B	0.6%	0.7%	0.8%	0.8%	—	—
63-B	0.7%	0.7%	0.7%	0.8%	—	—

[0567] As can be seen in Tables 46(a), 46(b) and FIG. **21**, the EDTA-containing compositions showed reduced levels of aggregation at all tested EDTA concentrations as compared to a composition lacking EDTA, but having an acetate buffer and sodium chloride tonicifying agent after storage at 25° C. and 40° C. FIG. **21** graphically summarizes the reduction in percent aggregation for the compositions from Table 42.

Fragmentation Analysis:

[0568] The antibody compositions prepared according to Table 42 were stored at a temperature of 25° C. and 40° C. At weeks, 0 (initial), 4, 8, 13, 18 and 24, the 25° C. and 40° C. compositions were analyzed for total hydrolytic impurities (i.e., fragmentation) using reduced SDS-PAGE (rSDS-PAGE). The composition vials were aseptically sampled at each time point and loaded onto NuPAGE 4-12% bis-Tris gels with colloidal blue (Coomassie) stain. Gel reduction was achieved by use of the NuPAGE® reducing agent. The percentage impurity (i.e., fragmentation) of each sample band in the reduced gels was estimated by scanning on either a Molecular Dynamics Personal Densitometer PDQC-90 or Bio-Rad GS800 Imaging Densitometer. Fragmentation level was calculated as a percentage of total band volume (see Tables 47(a) and 47(b)).

TABLE 47(a)

No.	Initial	4 weeks 25° C.	8 weeks 25° C.	13 weeks 25° C.	18 weeks 25° C.	24 weeks 25° C.
	Percent Fragmentation for Compositions in Table 42 after Storage at 25° C.:					
26-B	1.3%	—	—	3.3%	3.7%	—
55-B	1.6%	—	—	2.9%	—	—
56-B	1.6%	—	—	2.5%	—	—
57-B	1.6%	—	—	2.4%	—	2.9%
58-B	1.0%	—	—	2.5%	—	—
59-B	1.1%	—	—	2.6%	—	—
60-B	1.2%	—	—	2.6%	—	—
61-B	1.1%	—	—	2.5%	—	—
62-B	1.0%	—	—	2.5%	—	—
63-B	1.1%	2.3%	2.1%	2.6%	—	—

[0569] Table 47(b) below reports the fragmentation data that is graphically presented in FIG. **22**.

TABLE 47(b)

No.	Initial	4 weeks 40° C.	8 weeks 40° C.	13 weeks 40° C.	18 weeks 40° C.	24 weeks 40° C.
	Percent Fragmentation for Compositions in Table 42 after Storage at 40° C.:					
26-B	—	—	6.2%	7.3%	8.7%	10.1%
55-B	—	1.6%	2.3%	5.7%	—	—
56-B	—	1.9%	2.3%	4.8%	—	—
57-B	—	2.0%	2.6%	4.7%	—	5.3%
58-B	—	—	3.7%	5.6%	—	—
59-B	—	—	3.6%	5.7%	—	—
60-B	—	—	3.5%	5.3%	—	—
61-B	—	2.5%	3.3%	5.5%	—	—
62-B	—	2.6%	3.6%	5.9%	—	—
63-B	—	2.6%	3.6%	5.3%	—	—

[0570] As can be seen in Tables 47(a), 47(b) and FIG. **22**, the EDTA-containing compositions showed reduced levels of fragmentation as compared to a composition lacking EDTA, but having an acetate buffer and sodium chloride, after storage at 25° C. and 40° C.

EXAMPLE 13-B

[0571] This example illustrates the production of a liquid pharmaceutical composition containing anti-CTLA-4 antibody ticilimumab, L-histidine monohydrochloride monohydrate, disodium ethylenediaminetetraacetic acid dihydrate, α α-trehalose dihydrate, and polysorbate 80.

[0572] A liquid pharmaceutical composition of the present invention was formed by obtaining the following components: anti-CTLA-4 antibody ticilimumab (available from hybridoma cell line 11.2.1.4 deposited under ATCC Accession No. PTA-5169 according to Example 1 or recombinantly prepared from a mammalian cell line according to Example 2), L-histidine monohydrochloride monohydrate (available from Ajinomoto, Raleigh, N.C.), L-histidine (available from Ajinomoto, Raleigh, N.C.), disodium ethylenediaminetetraacetic acid dihydrate (available as Titriplex III from Merck KgaA, Darmstadt, Germany), α α-trehalose dihydrate (available as Product Number T-104-1-MC, from Ferro Pfanstiehl, Waukegan Ill.), and polysorbate 80 (available as Crillet 4 HP, from Croda Inc., Mill Hall Pa.).

[0573] The liquid pharmaceutical composition was prepared by first preparing several stock solutions of anti-CTLA-4 antibody ticilimumab, L-histidine monohydrochloride monohydrate, disodium ethylenediaminetetraacetic acid dihydrate, α α-trehalose dihydrate, and polysorbate 80. A 20 mM histidine buffer pH 5.5 is prepared by dissolving 3.27 mg/mL (15.6 mM) L-Histidine HCl monohydrate and 0.68 mg/mL (4.4 mM) L-Histidine in water. A 1× Composition buffer is prepared by dissolving 3.27 mg/mL (15.6 mM) L-Histidine HCl monohydrate and 0.68 mg/mL (4.4 mM) L-Histidine, 84 mg/mL (222 mM) α α-trehalose dihydrate, 0.2 mg/mL Polysorbate 80 and 0.1 mg/mL (0.268 mM) disodium ethylenediaminetetraacetic acid dihydrate in water. A 2× Composition buffer is prepared by dissolving 3.27 mg/mL (15.6 mM) L-Histidine HCl monohydrate and 0.68 mg/mL (4.4 mM) L-Histidine, 168 mg/mL (444 mM) α α-trehalose dihydrate, 0.4 mg/mL Polysorbate 80 and 0.2 mg/mL (0.536 mM) disodium ethylenediaminetetraacetic acid dihydrate in water. A stock solution of anti-CTLA-4 antibody ticilimumab is prepared according to Example 2 and concentrated to between 42 and 55 mg/ml (target 45 mg/mL) in the Histidine buffer using an ultrafiltration process carried out with a membrane Type 50 kD (Biomax PES).

[0574] To prepare the pharmaceutical composition, equal volumes of the concentrated stock solution of anti-CTLA-4 antibody ticilimumab and the 2× Composition buffer are added to a container suitable for intimate mixing of liquid compositions. After mixing, a small volume of the solution is removed and antibody concentration determined by Ultraviolet-Visible spectrometry (UV-Vis) method using an extinction coefficient of 1.43 (mg/mL)-1 cm^{-1} (expected range 21 to 27.5 mg/mL, target 22.5 mg/mL). Finally, an appropriately calculated volume of 1× Composition buffer is added and mixed to bring the antibody to the target concentration of 20 mg/mL (range 18-22 mg/mL).

[0575] The pharmaceutical compositions is then filtered through 0.2μ sterilizing grade filters and filled into vials. A nominal fill-volume of 20 milliliter was used in 20 milliliter Type 1 glass vials. The vials were closed with Dalkyo 777-1 Fluorotec® coated stoppers and crimp sealed. The glass vials were sterilized as were the 20 mm Dalkyo 777-1 serum stoppers.

[0576] Each single vial unit contains about 400 mg of anti-CTLA-4 antibody ticilimumab, 65.4 mg of L-histidine monohydrochloride monohydrate, 13.6 mg of L-Histidine, 2 mg of disodium ethylenediaminetetraacetic acid dihydrate, 1680 mg of α α-trehalose dihydrate, and 4 mg of polysorbate 80.

EXAMPLE 14-B

[0577] This example illustrates the prospective production of a liquid pharmaceutical composition containing anti-CTLA-4 antibody ticilimumab, L-histidine monohydrochloride monohydrate, calcium disodium ethylenediaminetetraacetic acid, α α-trehalose dihydrate, and polysorbate 80.

[0578] A liquid pharmaceutical composition of the present invention may be formed by obtaining the following components: anti-CTLA-4 antibody ticilimumab (available from hybridoma cell line 11.2.1.4 deposited under ATCC Accession No. PTA-5169 according to Example 1 or recombinantly prepared from a mammalian cell line according to Example 2-B), L-histidine monohydrochloride monohydrate (available from Ajinomoto, Raleigh, N.C.), L-histidine (available from Ajinomoto, Raleigh, N.C.), calcium disodium ethylenediaminetetraacetic acid (available from Sigma-Aldrich, St. Louis, Mo.), α α-trehalose dihydrate (available as Product

Number T-104-1-MC, from Ferro Pfanstiehl, Waukegan Ill.), and polysorbate 80 (available as Crillet 4 HP, from Croda Inc., Mill Hall Pa.).

[0579] The liquid pharmaceutical composition may be prepared by first preparing several stock solutions of anti-CTLA-4 antibody ticilimumab, L-histidine monohydrochloride monohydrate, disodium ethylenediaminetetraacetic acid dihydrate, α α-trehalose dihydrate, and polysorbate 80. A 20 mM histidine buffer pH 5.5 may be prepared by dissolving 3.27 mg/mL (15.6 mM) L-Histidine HCl monohydrate and 0.68 mg/mL (4.4 mM) L-Histidine in water. A 1× Composition buffer may be prepared by dissolving 3.27 mg/mL (15.6 mM) L-Histidine HCl monohydrate and 0.68 mg/mL (4.4 mM) L-Histidine, 84 mg/mL (222 mM) α α-trehalose dihydrate, 0.2 mg/mL Polysorbate 80 and 0.1003 mg/mL (0.268 mM) calcium disodium ethylenediaminetetraacetic acid in water. A 2× Composition buffer may be prepared by dissolving 3.27 mg/mL (15.6 mM) L-Histidine HCl monohydrate and 0.68 mg/mL (4.4 mM) L-Histidine, 168 mg/mL (444 mM) αα-trehalose dihydrate, 0.4 mg/mL Polysorbate 80 and 0.2006 mg/mL (0.536 mM) calcium disodium ethylenediaminetetraacetic acid in water. A stock solution of anti-CTLA-4 antibody ticilimumab may be prepared according to Example 2-B and concentrated to between 42 and 55 mg/ml (target 45 mg/mL) in the Histidine buffer using an ultrafiltration process carried out with a membrane Type 50 kD (Biomax PES).

[0580] To prepare the pharmaceutical composition, equal volumes of the concentrated stock solution of anti-CTLA-4 antibody ticilimumab and the 2× Composition buffer may be added to a container suitable for intimate mixing of liquid compositions. After mixing, a small volume of the solution may be removed and antibody concentration determined by Ultraviolet-Visible spectrometry (UV-Vis) method using an extinction coefficient of 1.43 $(mg/mL)^{-1}$ cm^{-1}, (expected range 21 to 27.5 mg/mL, target 22.5 mg/mL). Finally, an appropriately calculated volume of 1× Composition buffer may be added and mixed to bring the antibody to the target concentration of 20 mg/mL (range 18-22 mg/mL).

[0581] The pharmaceutical compositions may then be filtered through 0.2μ sterilizing grade filters and filled into vials. A nominal fill-volume of 20 milliliter may be used in 20 milliliter Type 1 glass vials. The vials may then be closed with Dalkyo 777-1 Fluorotec® coated stoppers and crimp sealed. The glass vials may be sterilized as well as the 20 mm Dalkyo 777-1 serum stoppers.

[0582] Each single vial unit would contain about 400 mg of anti-CTLA-4 antibody ticilimumab, 65.4 mg of L-histidine monohydrochloride monohydrate, 13.6 mg of L-Histidine, 2.006 mg of calcium disodium ethylenediaminetetraacetic acid, 1680 mg of a α-trehalose dihydrate, and 4 mg of polysorbate 80.

EXAMPLE 15-B

[0583] This example illustrates the prospective production of a liquid pharmaceutical composition containing anti-CTLA-4 antibody ticilimumab, L-histidine monohydrochloride monohydrate, trisodium ethylenediaminetetraacetic acid, α α-trehalose dihydrate, and polysorbate 80.

[0584] A liquid pharmaceutical composition of the present invention was formed by obtaining the following components: anti-CTLA-4 antibody ticilimumab (available from hybridoma cell line 11.2.1.4 deposited under ATCC Accession No. PTA-5169 according to Example 1 or recombinantly prepared from a mammalian cell line according to Example 2-B), L-histidine monohydrochloride monohydrate (available from Ajinomoto, Raleigh, N.C.), L-histidine (available from Ajinomoto, Raleigh, N.C.), trisodium ethylenediaminetetraacetic acid (available from Sigma-Aldrich, St. Louis, Mo.), α α-trehalose dihydrate (available as Product Number T-104-1-MC, from Ferro Pfanstiehl, Waukegan Ill.), and polysorbate 80 (available as Crillet 4 HP, from Croda Inc., Mill Hall Pa.).

[0585] The liquid pharmaceutical composition was prepared by first preparing several stock solutions of anti-CTLA-4 antibody ticilimumab, L-histidine monohydrochloride monohydrate, trisodium ethylenediaminetetraacetic, acid, α α-trehalose dihydrate, and polysorbate 80. A 20 mM histidine buffer pH 5.5 is prepared by dissolving 3.27 mg/mL (15.6 mM) L-Histidine HCl monohydrate and 0.68 mg/mL (4.4 mM) L-Histidine in water. A 1× Composition buffer is prepared by dissolving 3.27 mg/mL (15.6 mM) L-Histidine HCl monohydrate and 0.68 mg/mL (4.4 mM) L-Histidine, 84 mg/mL (222 mM) α α-trehalose dihydrate, 0.2 mg/mL Polysorbate 80 and 0.096 mg/mL (0.268 mM) trisodium ethylenediaminetetraacetic acid in water. A 2× Composition buffer is prepared by dissolving 3.27 mg/mL (15.6 mM) L-Histidine HCl monohydrate and 0.68 mg/mL (4.4 mM) L-Histidine, 168 mg/mL (444 mM) α α-trehalose dihydrate, 0.4 mg/mL Polysorbate 80 and 0.192 mg/mL (0.536 mM) trisodium ethylenediaminetetraacetic acid in water. A stock solution of anti-CTLA-4 antibody ticilimumab is prepared according to Example 2-B and concentrated to between 42 and 55 mg/ml (target 45 mg/mL) in the Histidine buffer using an ultrafiltration process carried out with a membrane Type 50 kD (Biomax PES).

[0586] To prepare the pharmaceutical composition, equal volumes of the concentrated stock solution of anti-CTLA-4 antibody ticilimumab and the 2× Composition buffer are added to a container suitable for intimate mixing of liquid compositions. After mixing, a small volume of the solution is removed and antibody concentration determined by Ultraviolet-Visible spectrometry (UV-Vis) method using an extinction coefficient of 1.43 (mg/mL)-1 cm^{-1} (expected range 21 to 27.5 mg/mL, target 22.5 mg/mL). Finally, an appropriately calculated volume of 1× Composition buffer is added and mixed to bring the antibody to the target concentration of 20 mg/mL (range 18-22 mg/mL).

[0587] The pharmaceutical compositions is then filtered through 0.2μ sterilizing grade filters and filled into vials. A nominal fill-volume of 20 milliliter was used in 20 milliliter Type 1 glass vials. The vials were closed with Dalkyo 777-1 Fluorotec® coated stoppers and crimp sealed. The glass vials were sterilized as were the 20 mm Dalkyo 777-1 serum stoppers.

[0588] Each single vial unit contains about 400 mg of anti-CTLA-4 antibody ticilimumab, 65.4 mg of L-histidine monohydrochloride monohydrate, 13.6 mg of L-Histidine, 1.92 mg of trisodium ethylenediaminetetraacetic acid, 1680 mg of α α-trehalose dihydrate, and 4 mg of polysorbate 80.

EXAMPLE 1-C

Generation of Anti-MAdCAM Producing Hybridomas

[0589] Antibodies of the invention were prepared in accordance with the present Example. Refer to PCT/US2005/000370.

Primary Immunogen Preparation

[0590] Two immunogens were prepared for immunisation of the XenoMouse™ mice: (i) a MAdCAM-IgG1 Fc fusion protein and (ii) cell membranes prepared from cells stably transfected with MAdCAM.

[0591] (i) MAdCAM-IgG_Fc Fusion Protein

Expression Vector Construction

[0592] An EcoRI/BglII cDNA fragment encoding the mature extracellular, immunoglobulin-like domain of MAdCAM was excised from a pINCY Incyte clone (3279276) and cloned into EcoRI/BamHI sites of the pIG1 vector (Simmons, D. L. (1993) in Cellular Interactions in Development: A Practical Approach, ed. Hartley, D. A. (Oxford Univ. Press, Oxford), pp. 93-127.)) to generate an in frame IgG, Fc fusion. The resulting insert was excised with EcoRI/NotI and cloned into pcDNA3.1+ (Invitrogen). The MAdCAM-IgG1 Fc cDNA in the vector was sequence confirmed. The amino acid sequence of the MAdCAM-IgG1 Fc fusion protein is shown below:

[0593] MAdCAM-IgG1 Fc Fusion Protein:

(SEQ ID NO: 5)

MDFGLALLLAGLLGLLLGQSLQVKPLQVEPPEPVVAVALGASRQLTCRLA

CADRGASVQWRGLDTSLGAVQSDTGRSVLTVRNASLSAAGTRVCVGSCGG

RTFQHTVQLLVYAFPDQLTVSPAALVPGDPEVACTAHKVTPVDPNALSFS

LLVGGQELEGAQALGPEVQEEEEEPQGDEDVLFRVTERWRLPPLGTPVPP

ALYCQATMRLPGLELSHRQAIPVLHSPTSPEPPDTTSPESPDTTSPESPD

TTSQEPPDTTSQEPPDTTSQEPPDTTSPEPPDKTSPEPAPQQGSTHTPRS

PGSTRTRRPEIQPKSCDKTHTCPPCPAPELLGGPSVFLFPPKPKDTLMIS

RTPEVTCWVDVSHEDPEVKFNWYVDGVEVHNAKTKPREEQYNSTYRWSVL

TVLHQDWLNGKEYKCKVSNKALPAPIEKTISKAKGQPREPQVYTLPPSRD

ELTKNQVSLTCLVKGFYPSDIAVEWESNGQPENNYKATPPVLDSDGSFFL

YSKLTVDKSRWQQGNVFSCSVMHEALHNHYTQKSLSLSPGK

Underlined: signal peptide
Bold: MAdCAM extracellular domain

Recombinant Protein Expression/Purification

[0594] CHO-DHFR cells were transfected with pcDNA3.1+ vector containing MAdCAM-IgG1 Fc fusion protein cDNA and stable clones expressing MAdCAM-IgG1 Fc fusion protein selected in Iscove's media containing 600 μg/mL G418 and 100 ng/mL methotrexate. For protein expression, a hollow fibre bioreactor was seeded with stably expressing MAdCAM-IgG1 Fc CHO cells in Iscove's media containing 10% low IgG fetal bovine serum (Gibco), non essential amino acids (Gibco), 2 mM glutamine (Gibco), sodium pyruvate (Gibco), 100 μg/mL G418 and 100 ng/mL methotrexate, and used to generate concentrated media supernatant. The MAdCAM-IgG, Fc fusion protein was purified from the harvested supernatant by affinity chromatography. Briefly, supernatant was applied to a HiTrap Protein G Sepharose (5 mL, Pharmacia) column (2 mL/min), washed with 25 mM Tris pH 8, 150 mM NaCl (5 column volumes) and eluted with 100 mM glycine pH 2.5 (1 mL/min), immediately neutralising fractions to pH 7.5 with 1M Tris pH 8. Fractions containing MAdCAM-IgG1 Fc fusion protein were identified by SDS-PAGE, pooled together and applied to a Sephacryl S100 column (Pharmacia), pre-equilibrated with 35 mM BisTris pH 6.5, 150 mM NaCl. The gel filtration was performed at 0.35 mL/min, collecting a peak of MAdCAM-IgG1 Fc fusion protein in ca. 3×5 mL fractions. These samples were pooled and applied to a Resource Q (6 mL, Pharmacia) column, pre-equilibrated in 35 mM BisTris pH6.5. The column was washed with 5 column volumes of 35 mM Bis Tris pH 6.5, 150 mM NaCl (6 mL/min) and MAdCAM-IgG, Fc fusion protein eluted into a 4-6 mL fraction with 35 mM Bis Tris pH 6.5, 400 mM NaCl. At this stage the protein was 90% pure and migrating as a single band at approximately 68 kD by SDS-PAGE. For use as an immunogen and all subsequent assays, the material was buffer exchanged into 25 mM HEPES pH 7.5, 1 mM EDTA, 1 mM DTT, 100 mM NaCl, 50% glycerol and stored as aliquots at −80° C.

[0595] (ii) Cell Membranes Stably Expressing MAdCAM

[0596] A SacI/NotI fragment comprising nucleotides 645-1222 of the published MAdCAM sequence (Shyjan A M, et al., J. Immunol., 156, 2851-7 (1996)) was PCR amplified from a colon cDNA library and cloned into SacI/NotI sites of pIND-Hygro vector (Invitrogen). A SacI fragment, comprising the additional 5' coding sequence was sub-cloned into this construct from pcDNA3.1 MAdCAM-IgG_1 Fc, to generate the full length MAdCAM cDNA. A KpnI/NotI fragment containing the MAdCAM cDNA was then cloned into corresponding sites in a pEF5FRTV5GWCAT vector (Invitrogen) and replacing the CAT coding sequence. The cDNA insert was sequence verified and used in transfections to generate single stably expressing clones in Flpln NIH 3T3 cells (Invitrogen) by Flp recombinase technology, according to the manufacturer's instructions. Stably expressing clones were selected by their ability to support the binding of a $\alpha_4\beta_7^+$ JY human B lymphoblastoid cell line (Chan B M, et al, J. Biol. Chem., 267:8366-70 (1992)), outlined below. Stable clones of CHO cells expressing MAdCAM were prepared in the same way, using Flpln CHO cells (Invitrogen).

[0597] MAdCAM-expressing Flpln NIH-3T3 cells were grown in Dulbecco's modified Eagles Medium (Gibco), containing 2 mM L-glutamine, 10% Donor calf serum (Gibco) and 200 μg/mL Hygromycin B (Invitrogen) and expanded in roller bottles. MAdCAM-expressing Flpln CHO cells were grown in Ham's F12/Dulbecco's modified Eagles Medium (Gibco), containing 2 mM L-glutamine, 10% Donor calf serum (Gibco) and 350 μg/mL Hygromycin B (Invitrogen) and expanded in roller bottles. Cells were harvested by use of a non-enzymatic cell dissociation solution (Sigma) and scraping, washing in phosphate buffered saline by centrifugation. Cell membranes were prepared from the cell pellet by two rounds of polytron homogenization in 25 mM Bis Tris pH 8, 10 mM $MgCl_2$, 0.015% (w/v) aprotinin, 100 U/mL bacitracin and centrifugation. The final pellet was resuspended in the same buffer, and 50×10^6 cell equivalents aliquoted into thick-walled eppendorfs and spun at >100,000 g to generate cell membrane pellets for XenoMouse mice immunisations. Supernatant was decanted and membranes were stored in eppendorfs at −80° C. until required. Confirmation of protein expression in the cell membranes was determined by SDS-PAGE and Western blotting with a rabbit anti-peptide antibody raised against the N-terminal residues of MAdCAM ([C]-KPLQVEPPEP).

Immunization and Hybridoma Generation

[0598] Eight to ten week old XENOMOUSE™ mice were immunized intraperitoneally or in their hind footpads with either the purified recombinant MAdCAM-IgG1 Fc fusion protein (10 μg/dose/mouse), or cell membranes prepared

from either stably expressing MAdCAM-CHO or NIH 3T3 cells (10×10^6 cells/dose/mouse). This dose was repeated five to seven times over a three to eight week period. Four days before fusion, the mice received a final injection of the extracellular domain of human MAdCAM in PBS. Spleen and lymph node lymphocytes from immunized mice were fused with the non-secretory myeloma P3-X63-Ag8.653 cell line and were subjected to HAT selection as previously described (Galfre and Milstein, Methods Enzymol. 73:3-46 (1981)). A panel of hybridomas secreting MAdCAM specific human IgG_2K was recovered and sub-cloned.

[0599] The following hybridoma producing anti-MAdCAM antibodies designated as follows was deposited at the European Collection of cell Cultures (ECACC), H.P.A. at CAMR, Porton Down, Salisbury, Wiltshire SP4 0JG on 9 Sep. 2003: Hybridoma 7.16.6, Deposit No. 03090909.

EXAMPLE 2-C

Antibody Compositions

[0600] The following compositions are referred to in the Examples that follow:

TABLE 47

Composition ID	mAb 7.16.6 mg/ml	Buffer mM; pH	Tonicifier mg/ml	Surfactant mg/ml	Chelating agent Mg/ml	Other Excipients
1-C	10 ± 1	Acetate, 20 mM, pH 5.5				
2-C	10 ± 1	Acetate, 7 mM Citrate, 7 mM Phosphate, 7 mM, pH 5.5				
3-C	10 ± 1	EDTA, 20 mM pH 5.5				
4-C	8 ± 1	Sodium acetate, 20 mM, pH 5.5				NaCl, 140 mM
5-C	8 ± 1	Sodium acetate, 20 mM, pH 5.5	Mannitol, 45 mg/ml			
6-C	8 ± 1	Sodium acetate, 10 mM, pH 5.5				
7-C	8 ± 1	Sodium EDTA, 10 mM, pH 5.5				
8-C	8 ± 1	Sodium EDTA, 10 mM, pH 5.5				NaCl, 140 mM
9-C	8 ± 1	Sodium EDTA, 10 mM, pH 5.5	Mannitol, 45 mg/ml			
10-C	8 ± 2	Sodium acetate, 20 mM, pH 5.5	Mannitol, 45 mg/ml	PS80, 0.2 mg/ml		
11-C	8 ± 2	Sodium acetate 20 mM, pH 5.5	Mannitol, 45 mg/ml	PS80, 0.2 mg/ml	$Na_2EDTA\bullet2H_20$, 0.02 mg/ml	
12-C	8 ± 2	Sodium acetate 20 mM, pH 5.5	NaCl, 8.2 mg/ml	PS80, 0.2 mg/ml		
13-C	8 ± 2	Sodium acetate 20 mM, pH 5.5	Mannitol, 45 mg/ml	PS80, 0.2 mg/ml	$CaCl_2\bullet2H_2O$, 0.3 mg/ml	
14-C	30 ± 6	Sodium acetate 20 mM, pH 5.5	Mannitol, 45 mg/ml	PS80, 0.4 mg/ml		

TABLE 47-continued

Composition ID	mAb 7.16.6 mg/ml	Buffer mM; pH	Tonicifier mg/ml	Surfactant mg/ml	Chelating agent Mg/ml	Other Excipients
15-C	30 ± 6	Sodium acetate 20 mM, pH 5.5	Mannitol, 45 mg/ml	PS80, 0.4 mg/ml	$Na_2EDTA•2H_20$, 0.02 mg/ml	
16-C	30 ± 6	Sodium acetate 20 mM, pH 5.5	NaCl, 8.2 mg/ml	PS80, 0.4 mg/ml		
17-C	30 ± 6	Sodium acetate 20 mM, pH 5.5	Mannitol, 45 mg/ml	PS80, 0.4 mg/ml	$CaCl_2•2H_2O$, 0.3 mg/ml	
18-C	50 ± 6	Histidine, 10 mM, pH 5.5	Mannitol, 45 mg/ml	PS80, 0.2 mg/ml	$Na_2EDTA•2H_20$, 0.02 mg/ml	
19-C	10 ± 2	Sodium acetate 20 mM, pH 5.5	Mannitol, 45 mg/ml	PS80, 0.2 mg/ml	$Na_2EDTA•2H_20$, 0.02 mg/ml	
20-C	10 ± 2	Histidine, 10 mM, pH 5.5	Mannitol, 45 mg/ml	PS80, 0.2 mg/ml	$Na_2EDTA•2H_20$, 0.02 mg/ml	
21-C	10 ± 2	Histidine, 10 mM, pH 5.5	Mannitol, 45 mg/ml	PS80, 0.2 mg/ml	$Na_2EDTA•2H_20$, 0.02 mg/ml	
22-C	50 ± 6	Sodium acetate 20 mM, pH 5.5	Mannitol, 45 mg/ml	PS80, 0.4 mg/ml	$Na_2EDTA•2H_20$, 0.02 mg/ml	
23-C	50 ± 6	Histidine, 10 mM, pH 6.0	Mannitol, 45 mg/ml	PS80, 0.2 mg/ml	$Na_2EDTA•2H_20$, 0.02 mg/ml	
24-C	150 ± 6	Histidine, 10 mM, pH 5.5	Mannitol, 45 mg/ml	PS80, 0.2 mg/ml	$Na_2EDTA•2H_20$, 0.02 mg/ml	
25-C	50 ± 5	Histidine, 10 mM, pH 5.5	Trehalose, 90 mg/ml	PS80, 0.2 mg/ml	$Na_2EDTA•2H_20$, 0.05 mg/ml	
26-C	75 ± 5	Histidine, 10 mM, pH 5.5	Trehalose, 90 mg/ml	PS80, 0.2 mg/ml	$Na_2EDTA•2H_20$, 0.05 mg/ml	
27-C	100 ± 7	Histidine, 10 mM, pH 5.5	Trehalose, 90 mg/ml	PS80, 0.2 mg/ml	$Na_2EDTA•2H_20$, 0.05 mg/ml	
28-C	150 ± 10	Histidine, 10 mM, pH 5.5	Trehalose, 90 mg/ml	PS80, 0.2 mg/ml	$Na_2EDTA•2H_20$, 0.05 mg/ml	
29-C	190 ± 2	Histidine, 10 mM, pH 5.5	Trehalose, 90 mg/ml	PS80, 0.2 mg/ml	$Na_2EDTA•2H_20$, 0.05 mg/ml	
30-C	80 ± 10	Histidine, 10 mM, pH 5.5	Trehalose, 90 mg/ml	PS80, 0.2 mg/ml	$Na_2EDTA•2H_20$, 0.02 mg/ml	
31-C	80 ± 10	Histidine, 10 mM, pH 5.5	Trehalose, 90 mg/ml	PS80, 0.2 mg/ml	$Na_2EDTA•2H_20$, 0.10 mg/ml	
32-C	80 ± 10	Histidine, 10 mM, pH 5.5	Trehalose, 90 mg/ml	PS80, 0.4 mg/ml	$Na_2EDTA•2H_20$, 0.05 mg/ml	
33-C	80 ± 10	Histidine, 10 mM, pH 5.5	Trehalose, 90 mg/ml	PS80, 1.0 mg/ml	$Na_2EDTA•2H_20$, 0.05 mg/ml	
34-C	85 ± 15	Histidine, 10 mM, pH 5.5	Trehalose, 90 mg/ml	PS80, 0.2 mg/ml	$Na_2EDTA•2H_20$, 0.10 mg/ml	
35-C	85 ± 15	Histidine, 10 mM, pH 5.5	Trehalose, 90 mg/ml	PS80, 0.4 mg/ml	$Na_2EDTA•2H_20$, 0.10 mg/ml	

TABLE 47-continued

Composition ID	mAb 7.16.6 mg/ml	Buffer mM; pH	Tonicifier mg/ml	Surfactant mg/ml	Chelating agent Mg/ml	Other Excipients
36-C	85 ± 15	Histidine, 10 mM, pH 5.5	Trehalose, 90 mg/ml	PS80, 1.0 mg/ml	$Na_2EDTA•2H_20$, 0.10 mg/ml	
37-C	80 ± 10	Citrate, 5 mM, pH 5.5	Trehalose, 90 mg/ml	PS80, 0.2 mg/ml	$Na_2EDTA•2H_20$, 0.05 mg/ml	
38-C	80 ± 10	Succinate, 5 mM pH 5.5	Trehalose, 90 mg/ml	PS80, 0.2 mg/ml	$Na_2EDTA•2H_20$, 0.05 mg/ml	
39-C	80 ± 10	Histidine, 10 mM, pH 5.5	Sucralose, 85 mg/ml	PS80, 0.2 mg/ml	$Na_2EDTA•2H_20$, 0.05 mg/ml	
40-C	80 ± 10	Histidine, 10 mM, pH 5.5	Sorbitol 45 mg/ml	PS80, 0.2 mg/ml	$Na_2EDTA•2H_20$, 0.05 mg/ml	
41-C	80 ± 10	Histidine, 10 mM, pH 5.5	Xylitol 35 mg/ml	PS80, 0.2 mg/ml	$Na_2EDTA•2H_20$, 0.05 mg/ml	
42-C	80 ± 10	Histidine, 10 mM, pH 5.5	Trehalose, 90 mg/ml	PEG_{3350}, 10 mg/ml	$Na_2EDTA•2H_20$, 0.05 mg/ml	
43-C	80 ± 10	Histidine, 10 mM, pH 5.5	Trehalose, 90 mg/ml	NOF PS80 0.2 mg/ml	$Na_2EDTA•2H_20$, 0.05 mg/ml	
44-C	80 ± 10	Histidine, 10 mM, pH 5.5	Trehalose, 90 mg/ml	Poloxamer 407, 1.0 mg/ml	$Na_2EDTA•2H_20$, 0.05 mg/ml	
45-C	80 ± 10	Histidine, 10 mM, pH 5.5	Trehalose, 90 mg/ml	Poloxamer 188 1.0 mg/ml	$Na_2EDTA•2H_20$, 0.05 mg/ml	
46-C	50 ± 5	Histidine, 10 mM, pH 5.5	Trehalose, 90 mg/ml	PS80, 0.2 mg/ml	$Na_2EDTA•2H_20$, 0.05 mg/ml	
47-C	50 ± 5	Histidine, 10 mM, pH 5.5	Trehalose, 90 mg/ml	PS80, 0.2 mg/ml	$Na_2EDTA•2H_20$, 0.05 mg/ml	
48-C	75 ± 15	Histidine, 10 mM, pH 5.5	Trehalose, 90 mg/ml	PS80, 0.4 mg/ml	$Na_2EDTA•2H_20$, 0.05 mg/ml	
49-C	75 ± 15	Histidine, 10 mM, pH 5.5	Trehalose, 90 mg/ml	PS80, 0.2 mg/ml	$Na_2EDTA•2H_20$, 0.05 mg/ml	
50-C	75 ± 15	Histidine, 10 mM, pH 5.5	Sucrose, 85 mg/ml	PS80, 0.4 mg/ml	$Na_2EDTA•2H_20$, 0.05 mg/ml	
51-C	75 ± 5	Histidine, 10 mM, pH 5.5	Trehalose, 90 mg/ml	PS80, 0.2 mg/ml	$Na_2EDTA•2H_20$, 0.05 mg/ml	
52-C	75 ± 5	Histidine, 10 mM, pH 5.5	Trehalose, 90 mg/ml	PS80, 0.4 mg/ml	$Na_2EDTA•2H_20$, 0.10 mg/ml	

EXAMPLE 3-C

[0601] A study was conducted to evaluate the effect of several different buffers on anti-MAdCAM antibody 7.16.6 aggregation.

Preparation of Buffer Solutions:

[0602] The buffer solutions were prepared by first dissolving an amount of the buffer species in water (approximately 90% of target). The pH of each buffer solutions was then adjusted to 5.5 by addition of a sufficient amount of an acid or base solution. After adjustment of pH, an additional amount of water was added to provide a final buffer concentration of 20 mM. The buffer concentration of 20 mM was selected to ensure reasonable pH stability at the selected pH of 5.5. The buffer solution was then filtered through a sterilization filter (0.22 micron pore size) into a sterilized receptacle for subsequent use.

Preparation of Antibody Compositions:

[0603] The antibody compositions were prepared as follows. An antibody bulk solution was obtained as 10.5 mg/ml in 20 mM sodium acetate buffer pH 5.5+140 mM sodium chloride. Buffer exchanges of this bulk solution into the composition solutions were carried out by centrifugation at 4500×g using a molecular weight cut-off membrane (e.g. 30 kD). Approximately 8 volume exchanges were made and the final antibody solution was prepared at about 10 mg/ml concentration. Antibody concentrations were determined by Ultraviolet-Visible spectrometry (UV-Vis) method using an

extinction coefficient of 1.56 (mg/ml)-1 cm^{-1} at 280 nm. The compositions with all ingredients were then sterilized by filtration through sterile 0.22 micron membrane filter. The filtered compositions were then filled into washed and autoclaved vials, which were closed with Dalkyo777-1 Fluorotec® coated stoppers, crimp sealed and placed in stability chambers.

[0604] Specifically, three liquid compositions comprising anti-MAdCAM antibody 7.16.6 and buffered with acetate, EDTA, or a combination of acetate, citrate and phosphate were prepared. The compositions were then stored for 6 weeks at 40° C. and aggregation measurements were taken.

TABLE 48

Composition ID	7.16.6 mAb mg/mL	Description	% Aggregation	Relative increase in % Aggregation*
1-C	10 ± 1	20 mM Acetate pH 5.5	0.8	70
2-C	10 ± 1	Combination, pH 5.5 (acetate 7 mM, citrate 7 mM, phosphate 6 mM)	0.7	60
3-C	10 ± 1	20 mM EDTA, pH 5.5	0.5	40

*Relative increase in % Aggregation is calculated by: [{(% aggregation at 6 wk /40 C) − (% aggregation at initial)} * 100]/[% aggregation at initial]

Aggregation Analysis:

[0605] The antibody compositions were stored at 40° C. At six weeks, each composition was, analyzed for aggregation using size exclusion chromatography (SEC). The size exclusion chromatography was carried out using a TSK gel G3000SWXL-G2000SWXL column, 0.2M sodium phosphate, pH7 mobile phase, a flow rate of 0.7 mL/min, and UV detection at 214 nm. Aggregation levels were calculated by integrating the areas under the chromatogram peaks for each composition and reporting the integrated areas under the high molecular weight species peaks as a percentage of total peak area. As is shown in Table 48, the EDTA buffered composition showed the lowest level of aggregation and lowest relative increase in aggregation.

EXAMPLE 4-C

[0606] A study was conducted to evaluate the effect of buffer concentration and presence/absence of other excipients on anti-MAdCAM antibody 7.16.6 fragmentation.

[0607] The compositions shown in Table 49 were prepared by the methods described in Example 2-C and evaluated by the methods in Example 3-C.

TABLE 49

Composition ID	7.16.6 mAb mg/mL	Buffer	Excipient	% fragmentation
4-C	8 ± 1	20 mM Sodium acetate, pH 5.5	NaCl, 140 mM	0.9
5-C	8 ± 1	20 mM Sodium acetate, pH 5.5	Mannitol, 45 mg/ml	0.6
6-C	8 ± 1	10 mM Sodium acetate, pH 5.5	—	0.5
7-C	8 ± 1	10 mM Sodium EDTA, pH 5.5	—	0
8-C	8 ± 1	10 mM Sodium EDTA, pH 5.5	NaCl, 140 mM	0
9-C	8 ± 1	10 mM Sodium EDTA, pH 5.5	Mannitol, 45 mg/ml	0

[0608] As shown in Table 49, the presence of EDTA in the liquid compositions results in less LMM formation than in compositions containing no EDTA.

EXAMPLE 5-C

[0609] A study was conducted to assess the effect of EDTA, in liquid anti-MAdCAM antibody compositions, on aggregation and fragmentation.

[0610] The buffer solutions were prepared by the methods described in Example 3-C. The antibody compositions were prepared as follows. An antibody bulk solution was obtained as 9.6 mg/ml in 20 mM sodium acetate buffer pH 5.5. Buffer exchanges of this bulk solution into the composition solutions were carried out by centrifugation at 5000×g using a molecular weight cut-off membrane (e.g. 30 kD). Approximately 8 volume exchanges were made and the final antibody solution was prepared at about 8 mg/ml or about 30 mg/ml protein concentration. Antibody concentrations were determined by Ultraviolet-Visible spectrometry (UV-Vis) method using an extinction coefficient of 1.56 (mg/ml)-1 cm^{-1} at 280 nm. A concentrate solution of polysorbate 80 (PS80) (typically 20 mg/ml) was prepared by dilution and dissolution of PS80 by the appropriate buffer. The PS80 concentrate was then added to the antibody solutions to obtain the final compositions described. The compositions with all ingredients were then sterilized by filtration through sterile 0.22 micron membrane filter. The filtered compositions were then filled into washed, autoclaved vials. The vials were closed with Dalkyo 777-1 Fluorotec® coated stoppers, crimp sealed, and placed in stability chambers.

[0611] The compositions in Table 50 were stored at 40° C. for 26 weeks, and evaluated by the SEC method described in Example 3-C.

TABLE 50

Composition ID	7.16.6 mAb mg/mL	Buffer (pH 5.5)	Excipient	PS-80, mg/mL	Other excipients	% Aggregate	% fragmentation
10-C	8 ± 2	Sodium acetate, 20 mM, pH 5.5	Mannitol, 45 mg/mL	0.2	—	6.9	0.6
11-C	8 ± 2	Sodium acetate, 20 mM, pH 5.5	Mannitol, 45 mg/mL	0.2	$Na_2EDTA•2H_2O$, 0.02 mg/mL	3.7	0
12-C	8 ± 2	Sodium acetate, 20 mM, pH 5.5	NaCl, 8.2 mg/mL	0.2	—	6.0	0.5
13-C	8 ± 2	Sodium acetate, 20 mM, pH 5.5	Mannitol, 45 mg/mL	0.2	$CaCl_2•2H_2O$, 0.3 mg/mL	6.8	0.8
14-C	30 ± 6	Sodium acetate, 20 mM, pH 5.5	Mannitol, 45 mg/mL	0.4	—	7.2	0.2
15-C	30 ± 6	Sodium acetate, 20 mM, pH 5.5	Mannitol, 45 mg/mL	0.4	$Na_2EDTA•2H_2O$, 0.02 mg/mL	3.7	0
16-C	30 ± 6	Sodium acetate, 20 mM, pH 5.5	NaCl, 8.2 mg/mL	0.4	—	7.7	0.4
17-C	30 ± 6	Sodium acetate, 20 mM, pH 5.5	Mannitol, 45 mg/mL	0.4	$CaCl_2•2H_2O$, 0.3 mg/mL	9.8	0.7

[0612] The compositions in Table 51 were stored at 25° C. for 26 weeks and evaluated by the SEC method described in Example 3-C.

TABLE 51

Composition ID	7.16.6 mAb, mg/mL	Buffer	Excipient	PS-80, mg/mL	Other excipients	% Aggregate
14-C	30 ± 6	Sodium acetate, 20 mM, pH 5.5	Mannitol, 45 mg/mL	0.4	—	1.6
15-C	30 ± 6	Sodium acetate, 20 mM, pH 5.5	Mannitol, 45 mg/mL	0.4	$Na_2EDTA•2H_2O$, 0.02 mg/mL	0.7
18-C	50 ± 6	Histidine 10 mM, pH 5.5	Mannitol, 45 mg/mL	0.2	$Na_2EDTA•2H_2O$, 0.02 mg/mL	0.5

[0613] This example shows that the presence of EDTA in liquid composition results in less aggregation and less LMM species formation.

EXAMPLE 6-C

[0614] A study was conducted to compare the effect of acetate and histidine buffers on aggregation and fragmentation of anti-MAdCAM antibody 7.16.6. The compositions shown in Tables 52 and 53 were prepared by the methods described in Example 5-C. The compositions in Table 52 were stored at 40° C. for 26 weeks and analyzed by the SEC methods described in Example 3-C.

TABLE 52

Composition ID	7.16.6 mAb mg/mL	Buffer	pH	Excipient	PS-80, mg/mL	$Na_2EDTA•2H_2O$, mg/mL	% Aggregate	% Fragment
19-C	10 ± 2	Sodium acetate, 20 mM	5.5	Mannitol, 45 mg/mL	0.2	0.02	3.8	0
20-C	10 ± 2	Histidine 10 mM	6.0	Mannitol, 45 mg/mL	0.2	0.02	1.2	0
21-C	10 ± 2	Histidine 10 mM	5.5	Mannitol, 45 mg/mL	0.2	0.02	1.5	0
22-C	50 ± 6	Sodium acetate, 20 mM	5.5	Mannitol, 45 mg/mL	0.4	0.02	5.2	0
23-C	50 ± 6	Histidine 10 mM	6.0	Mannitol, 45 mg/mL	0.2	0.02	2.3	0
18-C	50 ± 6	Histidine 10 mM	5.5	Mannitol, 45 mg/mL	0.2	0.02	2.8	0.1

The compositions in Table 53 were stored at 25° C. for 52 weeks, and analysed by the SEC methods described in Example 3-C.

TABLE 53

Composition ID	7.16.6 mAb mg/mL	Buffer	pH	Excipient	PS-80, mg/mL	$Na_2EDTA•2H_2O$, mg/mL	% Aggregate	% Fragment
19-C	10 ± 2	Sodium acetate, 20 mM	5.5	Mannitol, 45 mg/mL	0.2	0.02	0.9	0
20-C	10 ± 2	Histidine 10 mM	6.0	Mannitol, 45 mg/mL	0.2	0.02	0.4	0
21-C	10 ± 2	Histidine 10 mM	5.5	Mannitol, 45 mg/mL	0.2	0.02	0.3	0
22-C	50 ± 6	Sodium acetate, 20 mM	5.5	Mannitol, 45 mg/mL	0.4	0.02	1.3	0
23-C	50 ± 6	Histidine 10 mM	6.0	Mannitol, 45 mg/mL	0.2	0.02	0.8	0
18-C	50 ± 6	Histidine 10 mM	5.5	Mannitol, 45 mg/mL	0.2	0.02	0.9	0

[0615] This example shows that the compositions using histidine as a buffer have less aggregate formation than the compositions using acetate at the same pH.

EXAMPLE 7-C

[0616] A study was conducted to evaluate the aggregation propensity of anti-MAdCAM antibody 7.16.6 in compositions at various antibody concentrations. In this study the compositions in Table 54 were prepared by the methods described in Example 5-C. The compositions in Table 54 were stored at 5° C., 25° C. or 40° C. for 26 weeks and then analyzed by the SEC methods described in Example 3-C.

[0617] As shown in Table 54, the propensity for aggregation increases with increasing concentrations of antibody. However, after storage for 26 weeks, the stabilizing effect of the compositions shown in Table 54 is demonstrated by the accelerated condition data (storage at 25° C. and 40° C.) that shows relatively low levels of aggregation with high concentration compositions.

EXAMPLE 8-C

[0618] A study was conducted to evaluate the aggregation propensity of anti-MAdCAM antibody 7.16.6 in compositions with various levels of EDTA. The compositions were prepared as described above in Example 5-C, except that the final antibody concentration was adjusted to 80±10 mg/ml. The compositions in Table 55 were stored for 26 weeks at 5° C. or 26° C. and then analyzed by SEC as described above.

TABLE 54

Composition ID	Buffer, mM	pH	mAb 7.16.6, mg/mL	$Trehalose•2H_2O$, mg/mL	$Na_2EDTA•2H_2O$, mg/mL	PS80, mg/mL	% Aggregation at 5 C.	% Aggregation at 25 C.	% Aggregation at 40 C.
25-C	His, 10	5.5	47	90	0.05	0.2	0.3	0.6	2.7
26-C	His, 10	5.5	75	90	0.05	0.2	0.4	0.7	3.6
27-C	His, 10	5.5	99	90	0.05	0.2	0.5	0.8	4.4
28-C	His, 10	5.5	145	90	0.05	0.2	0.7	1.2	6.0
29-C	His, 10	5.5	183	90	0.05	0.2	0.6	1.3	7.5

TABLE 55

Composition ID	Buffer, mM	pH	Trehalose•$2H_2O$, mg/mL	Na_2EDTA•$2H_2O$, mg/mL	PS80, mg/mL	% Aggregation at 5 C.	% Aggregation at 25 C.
30-C	His, 10	5.5	90	0.02	0.2	0.6	0.9
26-C	His, 10	5.5	90	0.05	0.2	0.4	0.7
31-C	His, 10	5.5	90	0.10	0.2	0.5	0.8

[0619] As shown, there is an improvement at 0.05 mg/ml and 0.10 mg/mL relative to 0.02 mg/mL EDTA, and in each case, aggregation in the presence of EDTA is low after storage at 5° C. for 26 weeks.

EXAMPLE 9-C

[0620] A study was conducted to evaluate stability of compositions of anti-MAdCAM antibody 7.16.6 with various levels of Polysorbate 80. The compositions were prepared as described above, but with the final antibody concentration adjusted to 80± mg/ml. The compositions in Table 56 were stored for 26 weeks at 25° C. or 40° C. and then analyzed by SEC as described above.

TABLE 56

Composition ID	Buffer mM	pH	Trehalose•$2H_2O$, mg/mL	Na_2EDTA•$2H_2O$, mg/mL	PS80, mg/mL	% Aggregate at 25 C.	% Aggregation at 40 C.
26-C	His, 10	5.5	90	0.05	0.2	0.7	3.6
32-C	His, 10	5.5	90	0.05	0.4	0.8	4.2
33-C	His, 10	5.5	90	0.05	1.0	1.0	4.8

[0621] The compositions in Table 57 were subjected to shaking stress applied by orbital shaking at 300 rpm for 24 hours at ambient temperature. The compositions were prepared as described above, but with final antibody concentration adjusted to 85 mg/ml

TABLE 57

Composition ID	Buffer, mM	pH	Trehalose•$2H_2O$, mg/mL	Na_2EDTA•$2H_2O$, mg/mL	PS80, mg/mL	Appearance
34-C	His, 10	5.5	90	0.10	0.2	Presence of few particulates
35-C	His, 10	5.5	90	0.10	0.4	No particulates
36-C	His, 10	5.5	90	0.10	1.0	No particulates

[0622] Although the above-described storage stability study shows a slight increase in soluble aggregation levels with increasing levels of polysorbate-80, the shaking stress study indicates that a polysorbate-80 level of 0.4 mg/mL provides adequate protection from shaking stress.

EXAMPLE 10-C

[0623] A study was undertaken to evaluate aggregation propensity of anti-MAdCAM antibody 7.16.6 in compositions with various buffers. The compositions were prepared as described above, and adjusted to a final concentration of antibody of 80±10 mg/ml. The compositions in Table 58 were stored at 25° C. or 40° C. for 26 weeks.

TABLE 58

Composition ID	Buffer, mM	pH	Trehalose•2H$_2$O, mg/mL	Na$_2$EDTA•2H$_2$O, mg/mL	PS80, mg/mL	% Aggregate at 25 C.	% Aggregate at 40 C.
26-C	His, 10	5.5	90	0.05	0.2	0.7	3.6
37-C	Citrate, 5	5.5	90	0.05	0.2	1.7	6.0
38-C	Succinate, 5	5.5	90	0.05	0.2	1.0	4.9

[0624] The level of aggregation was lowest in the composition with histidine buffer.

EXAMPLE 11-C

[0625] A study was undertaken to evaluate aggregation propensity of anti-MAdCAM antibody 7.16.6 in compositions with various sugars and polyols. The compositions were prepared as described above, and adjusted to a final antibody concentration of 80±10 mg/ml. The compositions in Table 59 were stored at 40° C. for 26 weeks.

TABLE 59

Composition ID	Buffer, mM	pH	Sugar/Polyol, mg/mL	Na$_2$EDTA•2H$_2$O, mg/mL	PS80, mg/mL	% Aggregation at 40 C.
26-C	His, 10	5.5	Trehalose•2H$_2$O, 90	0.05	0.2	3.6
39-C	His, 10	5.5	Sucrose, 85	0.05	0.2	4.8
40-C	His, 10	5.5	Sorbitol, 45	0.05	0.2	4.8
41-C	His, 10	5.5	Xylitol, 35	0.05	0.2	4.6

[0626] The level of aggregation was lower with the composition containing trehalose.

EXAMPLE 12-C

[0627] A study was undertaken to evaluate antibody aggregation propensity of anti-MAdCAM antibody 7.16.6 in compositions with various surfactants and PEG. The compositions were prepared as described above, and adjusted to a final antibody concentration of 80±10 mg/ml. The final concentration of surfactant of PEG in the antibody compositions was achieved by addition of an appropriate quantity from concentrate stock solutions of surfactant or PEG. The compositions in Table 60 were stored at 40° C. for 26 weeks.

TABLE 60

Composition ID	Buffer, mM	pH	Trehalose•2H$_2$O, mg/mL	Na$_2$EDTA•2H$_2$O, mg/mL	Surfactant or PEG, mg/mL	% Aggregation at 40 C.
26-C	His, 10	5.5	90	0.05	PS80, 0.2	3.6
42-C	His, 10	5.5	90	0.05	PEG$_{3350}$, 10	3.9
43-C	His, 10	5.5	90	0.05	NOF PS80, 0.2	3.9
44-C	His, 10	5.5	90	0.05	Poloxamer 407, 1.0	3.4
45-C	His, 10	5.5	90	0.05	Poloxamer 188, 1.0	4.1

[0628] Compositions containing PS80 and Poloxamer 407 performed marginally better than the other surfactants and amphiphiles.

EXAMPLE 13-C

[0629] A study was undertaken to evaluate Met256 oxidation in compositions with either trehalose or sucurose. The compositions were prepared as described above, and adjusted to a final antibody concentration of 80±10 mg/ml. The compositions in Table 61 were stored at 5° C. or 40° C. for 26 weeks. Methionine oxidation was measured by enzymatically digesting the protein using Lysyl endoproteinase and the resulting peptide fragments were separated by reversed-phase HPLC with 214 nm absorbance detection. The peptide fragment containing methionine or its oxidized form was monitored. Percentage oxidation was calculated by peak area of oxidized methionine relative to that of parent methionine.

TABLE 61

Composition ID	Buffer, mM	pH	Sugar, mg/mL,	$Na_2EDTA•2H_2O$, mg/mL	PS80, mg/mL	% Met256 oxidation at 5 C.	% Met256 oxidation at 40 C.
26-C	His, 10	5.5	Trehalose•$2H_2O$, 90	0.05	0.2	3.3	7.2
32-C	His, 10	5.5	Trehalose•$2H_2O$, 90	0.05	0.4	3.2	7.3
39-C	His, 10	5.5	Sucrose, 85	0.05	0.2	3.2	9.0

[0630] The compositions containing trehalose show lower propensity for methionine oxidation relative to those containing sucrose.

EXAMPLE 14-C

[0631] A study was undertaken to evaluate the chemical stability performance of high antibody concentration compositions. The compositions in Tables 62 and 63 were prepared as described above, and adjusted to a final antibody concentration of 80±10 mg/ml. The compositions in Table 62 were stored at 5° C. for 26 weeks. Chemical stability was assessed by iCE. Measurements were conducted by preparing the protein mix with pl markers, methylcellulose, and pharmalytes to a final protein concentration of approximately 0.22 μg/μL. The electrophoresis run was performed with focusing time of 6 min. at 3000 volts and absorbance probe at 280 nm. Relative percentage of various charged species was determined by their respective area under the peak.

[0632] The compositions in Table 62 show good chemical stability, as no significant change in the major species as well as in total acidic species or total basic species.

[0633] The compositions in Table 63 were stored at 5° C. for 26 weeks, and assayed by reduced SDS-PAGE to determine purity. The SDS-PAGE gels were run using NuPAGE 4-12% Bis-Tris gel, and colloidal blue (Coomassie blue) stain. For the reduced gels, reduction was achieved by NuPAGE reducing agent. Percent purity in reduced gels was estimated densitometrically by: % purity=(% heavy chain+% light chain).

TABLE 62

Composition ID	Buffer, mM	pH	Trehalose•$2H_2O$, mg/mL	$Na_2EDTA•2H_2O$, mg/mL	PS80, mg/mL	% Major band by iCE (initial)	% Major band by iCE (26 weeks)
26-C	His, 10	5.5	90	0.05	0.2	66.5	67.9
32-C	His, 10	5.5	90	0.05	0.4	66.6	67.3
31-C	His, 10	5.5	90	0.10	0.2	65.7	65.5

TABLE 63

Composition ID	Buffer, mM	pH	Trehalose•$2H_2O$, mg/mL	$Na_2EDTA•2H_2O$, mg/mL	PS80, mg/mL	% Purity by reduced SDS-PAGE (initial)	% Purity by reduced SDS-PAGE (26 weeks)
26-C	His, 10	5.5	90	0.05	0.2	99.7	98.8
32-C	His, 10	5.5	90	0.05	0.4	99.7	98.8
31-C	His, 10	5.5	90	0.10	0.2	99.7	98.8

[0634] The compositions in Table 63 show no significant change in purity after storage for 26 weeks at 5° C., indicating good chemical stability.

EXAMPLE 15-C

[0635] A study was undertaken to evaluate the performance of high concentration compositions against freeze-thaw stress.

[0636] The compositions in Table 64 were prepared as described above, and adjusted to a final antibody concentration of 50±10 mg/ml. The compositions in Table 64 were subjected to three freeze-thaw cycles at −70° C./5° C. or −20° C./5° C. The studies were conducted in 2 ml glass vials with 1 ml fill. SE_HPLC measurements Were conducted using 0.2M sodium phosphate, pH 7 mobile phase, TSK gel G3000SWXL columns, at a flow rate of 0.7 ml/min, probe at 214 nm. Aggregate quantity was determined by summing antibody related peaks that eluted prior to the antibody monomer.

[0637] The compositions in Table 64 show no increase in aggregation after 3 freeze-thaw cycles at either −70° C./5° C. or −20° C./5° C.

[0638] The compositions in Table 65 were prepared as described above, and adjusted to a final antibody concentration of 75±15 mg/ml. The compositions in Table 65 were subjected to four freeze-thaw cycles at −20° C./5° C. These freeze-thaw studies were conducted in 10 ml glass vials with 10 ml fill. SEC measurements were conducted as indicated above in this example.

TABLE 64

Composition ID	Composition Description	Freeze-thaw cycle	Appearance after three freeze-thaw cycles	Change in % soluble aggregation after three freeze-thaw cycles
46-C	50 mg/mL mAb 7.16.6, 10 mM histidine, pH 5.5, 90 mg/mL trehalose dihydrate, 0.05 mg/mL disodium EDTA dihydrate, 0.2 mg/mL polysorbate 80	−70 C./5 C.	Clear; No particulates	0.0
47-C	50 mg/mL mAb 7.16.6, 10 mM histidine, pH 5.5, 90 mg/mL trehalose dihydrate, 0.05 mg/mL disodium EDTA dihydrate, 0.2 mg/mL polysorbate 80	−20 C./5 C.	Clear; No particulates	0.0

TABLE 65

Composition ID	Composition Description	Freeze-thaw cycle	Appearance after four freeze-thaw cycles	Change in % soluble aggregation after four freeze-thaw cycles
48-C	75 mg/mL mAb 7.16.6, 10 mM histidine, pH 5.5, 90 mg/mL trehalose dihydrate, 0.05 mg/mL disodium EDTA dihydrate, 0.4 mg/mL polysorbate 80	−20 C./5 C.	Clear; No particulates	0.0
49-C	75 mg/mL mAb 7.16.6, 10 mM histidine, pH 5.5, 90 mg/mL trehalose dihydrate,	−20 C./5 C.	Clear; No particulates	0.0

TABLE 65-continued

Composition ID	Composition Description	Freeze-thaw cycle	Appearance after four freeze-thaw cycles	Change in % soluble aggregation after four freeze-thaw cycles
50-C	0.05 mg/mL disodium EDTA dihydrate, 0.2 mg/mL polysorbate 80 75 mg/mL mAb 7.16.6, 10 mM histidine, pH 5.5, 85 mg/mL sucrose, 0.05 mg/mL disodium EDTA dihydrate, 0.4 mg/mL polysorbate 80	−20 C./5 C.	Clear; No particulates	0.0

[0639] The compositions in Table 65 show no increase in aggregation after four freeze-thaw cycles at −20° C./5° C.

EXAMPLE 16-C

[0640] A study was undertaken to assess the stability of a high concentration composition during frozen storage. The composition in Table 66 was prepared as described above, and final antibody concentration was adjusted to about 75 mg/ml. The composition in Table 21 was stored at −20° C. for 13 weeks, and aggregation assessed as described in Example 15-C.

TABLE 66

Composition ID	Buffer, mM	pH	Trehalose•$2H_2O$, mg/mL	$Na_2EDTA•2H_2O$, mg/mL	PS80, mg/mL	% Increase in aggregation at −20 C.
51-C	His, 10	5.5	90	0.05	0.2	0.0

[0641] The high concentration (75 mg/ml antibody) composition shows no increase in aggregation after 13 weeks of storage at −20° C.

EXAMPLE 17-C

[0642] A study was conducted to assess the viscosity of a high concentration composition. The composition in Table 67 was prepared as described above, and final antibody concentration was adjusted to about 75 mg/ml. Viscosity measurements were conducted by applying an average shear rate of 3005-1 to the composition placed on a rheometer plate.

TABLE 67

Composition 52-C:	mAb 7.16.6, mg/mL	Viscosity at 5 C., cP
10 mM histidine, pH 5.5, 90 mg/mL trehalose dihydrate, 0.10 mg/mL disodium EDTA dihydrate, 0.4 mg/mL polysorbate 80	75	5.7

[0643] The composition shows a viscosity suitable for subcutaneous administration.

[0644] All references cited in this specification, including without limitation all papers, publications, patents, patent applications, presentations, texts, reports, manuscripts, brochures, books, internet postings, journal articles, periodicals, and the like, are hereby incorporated by reference into this specification in their entireties. The discussion of the references herein is intended merely to summarize the assertions made by their authors and no admission is made that any reference constitutes prior art. Applicants reserve the right to challenge the accuracy and pertinency of the cited references.

[0645] As various changes could be made in the above methods and compositions without departing from the scope of the invention, it is intended that all matter contained in the above description shall be interpreted as illustrative and not in a limiting sense. In addition, it should be understood that aspects of the various embodiments may be interchanged both in whole or in part.

[0646] The compositions in Table 65 show no increase in aggregation after four freeze-thaw cycles at −20° C./5° C.

EXAMPLE 16-C

[0647] A study was undertaken to assess the stability of a high concentration composition during frozen storage. The composition in Table 66 was prepared as described above, and final antibody concentration was adjusted to about 75 mg/ml. The composition in Table 21 was stored at −20° C. for 13 weeks, and aggregation assessed as described in Example 15-C.

TABLE 66

Composition ID	Buffer, mM	pH	Trehalose•$2H_2O$, mg/mL	$Na_2EDTA•2H_2O$, mg/mL	PS80, mg/mL	% Increase in aggregation at −20 C.
51-C	His, 10	5.5	90	0.05	0.2	0.0

[0648] The high concentration (75 mg/ml antibody) composition shows no increase in aggregation after 13 weeks of storage at −20° C.

EXAMPLE 17-C

[0649] A study was conducted to assess the viscosity of a high concentration composition. The composition in Table 67 was prepared as described above, and final antibody concentration was adjusted to about 75 mg/ml. Viscosity measurements were conducted by applying an average shear rate of 300^{s-1} to the composition placed on a rheometer plate.

TABLE 67

Composition 52-C:	mAb 7.16.6, mg/mL	Viscosity at 5 C., cP
10 mM histidine, pH 5.5, 90 mg/mL trehalose dihydrate, 0.10 mg/mL disodium EDTA dihydrate, 0.4 mg/mL polysorbate 80	75	5.7

[0650] The composition shows a viscosity suitable for subcutaneous administration.

[0651] All references cited in this specification, including without limitation all papers, publications, patents, patent applications, presentations, texts, reports, manuscripts, brochures, books, internet postings, journal articles, periodicals, and the like, are hereby incorporated by reference into this specification in their entireties. The discussion of the references herein is intended merely to summarize the assertions made by their authors and no admission is made that any reference constitutes prior art. Applicants reserve the right to challenge the accuracy and pertinency of the cited references.

[0652] As various changes could be made in the above methods and compositions without departing from the scope of the invention, it is intended that all matter contained in the above description shall be interpreted as illustrative and not in a limiting sense. In addition, it should be understood that aspects of the various embodiments may be interchanged both in whole or in part.

SEQUENCE LISTING

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Pro Gly Gly Ser Leu Arg Leu Ser Cys Ala Ala Ser Gly Phe Thr Phe
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Glu Trp Val Ser Tyr Ile Ser Ser Arg Ser Ser Thr Ile Ser Tyr Ala
65 70 75 80

Asp Ser Val Lys Gly Arg Phe Thr Ile Ser Arg Asp Asn Ala Lys Asn
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Ser Leu Tyr Leu Gln Met Asn Ser Leu Arg Asp Glu Asp Thr Ala Val
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Tyr Tyr Cys Ala Arg Asp Pro Leu Leu Ala Gly Ala Thr Phe Phe Asp
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Tyr Trp Gly Gln Gly Thr Leu Val Thr Val Ser Ser Ala Ser Thr Lys
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Gly Pro Ser Val Phe Pro Leu Ala Pro Cys Ser Arg Ser Thr Ser Glu
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Ser Thr Ala Ala Leu Gly Cys Leu Val Lys Asp Tyr Phe Pro Glu Pro
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180 185 190

Phe Pro Ala Val Leu Gln Ser Ser Gly Leu Tyr Ser Leu Ser Ser Val
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        275                 280                 285

Asp Pro Glu Val Gln Phe Asn Trp Tyr Val Asp Gly Val Glu Val His
    290                 295                 300

Asn Ala Lys Thr Lys Pro Arg Glu Glu Gln Phe Asn Ser Thr Phe Arg
305                 310                 315                 320

Val Val Ser Val Leu Thr Val Val His Gln Asp Trp Leu Asn Gly Lys
                325                 330                 335

Glu Tyr Lys Cys Lys Val Ser Asn Lys Gly Leu Pro Ala Pro Ile Glu
            340                 345                 350

Lys Thr Ile Ser Lys Thr Lys Gly Gln Pro Arg Glu Pro Gln Val Tyr
        355                 360                 365

Thr Leu Pro Pro Ser Arg Glu Glu Met Thr Lys Asn Gln Val Ser Leu
    370                 375                 380

Thr Cys Leu Val Lys Gly Phe Tyr Pro Ser Asp Ile Ala Val Glu Trp
385                 390                 395                 400

Glu Ser Asn Gly Gln Pro Glu Asn Asn Tyr Lys Thr Thr Pro Pro Met
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Leu Asp Ser Asp Gly Ser Phe Phe Leu Tyr Ser Lys Leu Thr Val Asp
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Lys Ser Arg Trp Gln Gln Gly Asn Val Phe Ser Cys Ser Val Met His
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Val Ser Ser Ser Tyr Leu Ala Trp Tyr Gln Gln Lys Pro Gly Gln Ala
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Pro Arg Leu Leu Ile Tyr Gly Ala Ser Ser Arg Ala Thr Gly Ile Pro
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Asp Arg Phe Ser Gly Ser Gly Ser Gly Thr Asp Phe Thr Leu Thr Ile
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Ser Arg Leu Glu Pro Glu Asp Phe Ala Val Tyr Tyr Cys Gln Gln Tyr
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Gly Ser Ser Pro Leu Thr Phe Gly Gly Gly Thr Lys Val Glu Ile Lys
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Arg Thr Val Ala Ala Pro Ser Val Phe Ile Phe Pro Pro Ser Asp Glu
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Gln Leu Lys Ser Gly Thr Ala Ser Val Val Cys Leu Leu Asn Asn Phe
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Tyr Pro Arg Glu Ala Lys Val Gln Trp Lys Val Asp Asn Ala Leu Gln
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Ser Gly Asn Ser Gln Glu Ser Val Thr Glu Gln Asp Ser Lys Asp Ser
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Thr Tyr Ser Leu Ser Ser Thr Leu Thr Leu Ser Lys Ala Asp Tyr Glu
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Ala Val Ile Trp Tyr Asp Gly Ser Asn Lys Tyr Tyr Ala Asp Ser Val
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Lys Gly Arg Phe Thr Ile Ser Arg Asp Asn Ser Lys Asn Thr Leu Tyr
65 70 75 80

Leu Gln Met Asn Ser Leu Arg Ala Glu Asp Thr Ala Val Tyr Tyr Cys
85 90 95

Ala Arg Asp Pro Arg Gly Ala Thr Leu Tyr Tyr Tyr Tyr Tyr Gly Met
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Asp Val Trp Gly Gln Gly Thr Thr Val Thr Val Ser Ser Ala Ser Thr
115 120 125

Lys Gly Pro Ser Val Phe Pro Leu Ala Pro Cys Ser Arg Ser Thr Ser
130 135 140

Glu Ser Thr Ala Ala Leu Gly Cys Leu Val Lys Asp Tyr Phe Pro Glu
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Pro Val Thr Val Ser Trp Asn Ser Gly Ala Leu Thr Ser Gly Val His
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Thr Phe Pro Ala Val Leu Gln Ser Ser Gly Leu Tyr Ser Leu Ser Ser

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Gly Pro Ser Val Phe Leu Phe Pro Pro Lys Pro Lys Asp Thr Leu Met
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Ile Ser Arg Thr Pro Glu Val Thr Cys Val Val Val Asp Val Ser His
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Glu Asp Pro Glu Val Gln Phe Asn Trp Tyr Val Asp Gly Val Glu Val
        275                 280                 285

His Asn Ala Lys Thr Lys Pro Arg Glu Glu Gln Phe Asn Ser Thr Phe
    290                 295                 300

Arg Val Val Ser Val Leu Thr Val Val His Gln Asp Trp Leu Asn Gly
305                 310                 315                 320

Lys Glu Tyr Lys Cys Lys Val Ser Asn Lys Gly Leu Pro Ala Pro Ile
                325                 330                 335

Glu Lys Thr Ile Ser Lys Thr Lys Gly Gln Pro Arg Glu Pro Gln Val
            340                 345                 350

Tyr Thr Leu Pro Pro Ser Arg Glu Glu Met Thr Lys Asn Gln Val Ser
        355                 360                 365

Leu Thr Cys Leu Val Lys Gly Phe Tyr Pro Ser Asp Ile Ala Val Glu
    370                 375                 380

Trp Glu Ser Asn Gly Gln Pro Glu Asn Asn Tyr Lys Thr Thr Pro Pro
385                 390                 395                 400

Met Leu Asp Ser Asp Gly Ser Phe Phe Leu Tyr Ser Lys Leu Thr Val
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Asp Lys Ser Arg Trp Gln Gln Gly Asn Val Phe Ser Cys Ser Val Met
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Tyr Ala Ala Ser Ser Leu Gln Ser Gly Val Pro Ser Arg Phe Ser Gly
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Ser Gly Ser Gly Thr Asp Phe Thr Leu Thr Ile Ser Ser Leu Gln Pro
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Glu Asp Phe Ala Thr Tyr Tyr Cys Gln Gln Tyr Tyr Ser Thr Pro Phe
                85                  90                  95

Thr Phe Gly Pro Gly Thr Lys Val Glu Ile Lys Arg Thr Val Ala Ala
            100                 105                 110

Pro Ser Val Phe Ile Phe Pro Pro Ser Asp Glu Gln Leu Lys Ser Gly
        115                 120                 125

Thr Ala Ser Val Val Cys Leu Leu Asn Asn Phe Tyr Pro Arg Glu Ala
    130                 135                 140

Lys Val Gln Trp Lys Val Asp Asn Ala Leu Gln Ser Gly Asn Ser Gln
145                 150                 155                 160

Glu Ser Val Thr Glu Gln Asp Ser Lys Asp Ser Thr Tyr Ser Leu Ser
                165                 170                 175

Ser Thr Leu Thr Leu Ser Lys Ala Asp Tyr Glu Lys His Lys Val Tyr
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Leu Gln Met Asn Ser Leu Arg Ala Glu Asp Thr Ala Val Tyr Tyr Cys
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        35                  40                  45

Tyr Ala Ala Ser Ser Leu Gln Ser Gly Val Pro Ser Arg Phe Ser Gly
    50                  55                  60

Ser Gly Ser Gly Thr Asp Phe Thr Leu Thr Ile Ser Ser Leu Gln Pro
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<213> ORGANISM: Homo sapiens

<400> SEQUENCE: 12

Val Ile Trp Tyr Asp Gly Ser Asn Lys Tyr Tyr Ala Asp Ser Val
1               5                   10                  15


<210> SEQ ID NO 13
<211> LENGTH: 16
<212> TYPE: PRT
<213> ORGANISM: Homo sapiens

<400> SEQUENCE: 13

Asp Pro Arg Gly Ala Thr Leu Tyr Tyr Tyr Tyr Tyr Gly Met Asp Val
1               5                   10                  15
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<210> SEQ ID NO 14
<211> LENGTH: 11
<212> TYPE: PRT
<213> ORGANISM: Homo sapiens

<400> SEQUENCE: 14

Arg Ala Ser Gln Ser Ile Asn Ser Tyr Leu Asp
1 5 10

<210> SEQ ID NO 15
<211> LENGTH: 7
<212> TYPE: PRT
<213> ORGANISM: Homo sapiens

<400> SEQUENCE: 15

Ala Ala Ser Ser Leu Gln Ser
1 5

<210> SEQ ID NO 16
<211> LENGTH: 9
<212> TYPE: PRT
<213> ORGANISM: Homo sapiens

<400> SEQUENCE: 16

Gln Gln Tyr Tyr Ser Thr Pro Phe Thr
1 5

<210> SEQ ID NO 17
<211> LENGTH: 1410
<212> TYPE: DNA
<213> ORGANISM: Homo sapiens

<400> SEQUENCE: 17

atggactgga cctggagcat ccttttcttg gtggcagcag caacaggtgc ccactcccag 60

gttcagctgg tgcagtctgg agctgaggtg aagaagcctg gggcctcagt gaaggtctcc 120

tgcaaggctt ctggttacac ctttaccagc tatggtatca actgggtgcg acaggcccct 180

ggacaagggc ttgagtggat gggatggatc agcgtttaca gtggtaacac aaactatgca 240

cagaaggtcc agggcagagt caccatgacc gcagacacat ccacgagcac agcctacatg 300

gacctgagga gcctgagatc tgacgacacg gccgtgtatt actgtgcgag agagggtagc 360

agctcgtccg gagactacta ttacggtatg gacgtctggg gccaagggac cacggtcacc 420

gtctcctcag cctccaccaa gggcccatcg gtcttccccc tggcgccctg ctccaggagc 480

acctccgaga gcacagcggc cctgggctgc ctggtcaagg actacttccc cgaaccggtg 540

acggtgtcgt ggaactcagg cgctctgacc agcggcgtgc acaccttccc agctgtccta 600

cagtcctcag gactctactc cctcagcagc gtggtgaccg tgccctccag caacttcggc 660

acccagacct acacctgcaa cgtagatcac aagcccagca acaccaaggt ggacaagaca 720

gttgagcgca aatgttgtgt cgagtgccca ccgtgcccag caccacctgt ggcaggaccg 780

tcagtcttcc tcttcccccc aaaacccaag gacaccctca tgatctcccg gacccctgag 840

gtcacgtgcg tggtggtgga cgtgagccac gaagaccccg aggtccagtt caactggtac 900

gtggacggcg tggaggtgca taatgccaag acaaagccac gggaggagca gttcaacagc 960

acgttccgtg tggtcagcgt cctcaccgtt gtgcaccagg actggctgaa cggcaaggag 1020

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tacaagtgca aggtctccaa caaaggcctc ccagccccca tcgagaaaac catctccaaa 1080

accaaagggc agccccgaga accacaggtg tacaccctgc ccccatcccg ggaggagatg 1140

accaagaacc aggtcagcct gacctgcctg gtcaaaggct tctaccccag cgacatcgcc 1200

gtggagtggg agagcaatgg gcagccggag aacaactaca agaccacacc tcccatgctg 1260

gactccgacg gctccttctt cctctacagc aagctcaccg tggacaagag caggtggcag 1320

caggggaacg tcttctcatg ctccgtgatg catgaggctc tgcacaacca ctacacgcag 1380

aagagcctct ccctgtctcc gggtaaatga 1410

<210> SEQ ID NO 18
<211> LENGTH: 469
<212> TYPE: PRT
<213> ORGANISM: Homo sapiens

<400> SEQUENCE: 18

Met Asp Trp Thr Trp Ser Ile Leu Phe Leu Val Ala Ala Ala Thr Gly
1 5 10 15

Ala His Ser Gln Val Gln Leu Val Gln Ser Gly Ala Glu Val Lys Lys
20 25 30

Pro Gly Ala Ser Val Lys Val Ser Cys Lys Ala Ser Gly Tyr Thr Phe
35 40 45

Thr Ser Tyr Gly Ile Asn Trp Val Arg Gln Ala Pro Gly Gln Gly Leu
50 55 60

Glu Trp Met Gly Trp Ile Ser Val Tyr Ser Gly Asn Thr Asn Tyr Ala
65 70 75 80

Gln Lys Val Gln Gly Arg Val Thr Met Thr Ala Asp Thr Ser Thr Ser
85 90 95

Thr Ala Tyr Met Asp Leu Arg Ser Leu Arg Ser Asp Asp Thr Ala Val
100 105 110

Tyr Tyr Cys Ala Arg Glu Gly Ser Ser Ser Ser Gly Asp Tyr Tyr Tyr
115 120 125

Gly Met Asp Val Trp Gly Gln Gly Thr Thr Val Thr Val Ser Ser Ala
130 135 140

Ser Thr Lys Gly Pro Ser Val Phe Pro Leu Ala Pro Cys Ser Arg Ser
145 150 155 160

Thr Ser Glu Ser Thr Ala Ala Leu Gly Cys Leu Val Lys Asp Tyr Phe
165 170 175

Pro Glu Pro Val Thr Val Ser Trp Asn Ser Gly Ala Leu Thr Ser Gly
180 185 190

Val His Thr Phe Pro Ala Val Leu Gln Ser Ser Gly Leu Tyr Ser Leu
195 200 205

Ser Ser Val Val Thr Val Pro Ser Ser Asn Phe Gly Thr Gln Thr Tyr
210 215 220

Thr Cys Asn Val Asp His Lys Pro Ser Asn Thr Lys Val Asp Lys Thr
225 230 235 240

Val Glu Arg Lys Cys Cys Val Glu Cys Pro Pro Cys Pro Ala Pro Pro
245 250 255

Val Ala Gly Pro Ser Val Phe Leu Phe Pro Pro Lys Pro Lys Asp Thr
260 265 270

Leu Met Ile Ser Arg Thr Pro Glu Val Thr Cys Val Val Val Asp Val
275 280 285

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Ser His Glu Asp Pro Glu Val Gln Phe Asn Trp Tyr Val Asp Gly Val
    290                 295                 300

Glu Val His Asn Ala Lys Thr Lys Pro Arg Glu Glu Gln Phe Asn Ser
305                 310                 315                 320

Thr Phe Arg Val Val Ser Val Leu Thr Val Val His Gln Asp Trp Leu
                325                 330                 335

Asn Gly Lys Glu Tyr Lys Cys Lys Val Ser Asn Lys Gly Leu Pro Ala
            340                 345                 350

Pro Ile Glu Lys Thr Ile Ser Lys Thr Lys Gly Gln Pro Arg Glu Pro
        355                 360                 365

Gln Val Tyr Thr Leu Pro Pro Ser Arg Glu Glu Met Thr Lys Asn Gln
    370                 375                 380

Val Ser Leu Thr Cys Leu Val Lys Gly Phe Tyr Pro Ser Asp Ile Ala
385                 390                 395                 400

Val Glu Trp Glu Ser Asn Gly Gln Pro Glu Asn Asn Tyr Lys Thr Thr
                405                 410                 415

Pro Pro Met Leu Asp Ser Asp Gly Ser Phe Phe Leu Tyr Ser Lys Leu
            420                 425                 430

Thr Val Asp Lys Ser Arg Trp Gln Gln Gly Asn Val Phe Ser Cys Ser
        435                 440                 445

Val Met His Glu Ala Leu His Asn His Tyr Thr Gln Lys Ser Leu Ser
    450                 455                 460

Leu Ser Pro Gly Lys
465
```

<210> SEQ ID NO 19
<211> LENGTH: 723
<212> TYPE: DNA
<213> ORGANISM: Homo sapiens

<400> SEQUENCE: 19

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atgaggctcc ctgctcagct cctggggctg ctaatgctct ggatacctgg atccagtgca     60

gatattgtga tgacccagac tccactctct ctgtccgtca cccctggaca gccggcctcc    120

atctcctgca agtctagtca gagcctcctg catactgatg gaacgaccta tttgtattgg    180

tacctgcaga agccaggcca gcctccacag ctcctgatct atgaagtttc caaccggttc    240

tctggagtgc cagataggtt cagtggcagc gggtcaggga cagatttcac actgaaaatc    300

agccgggtgg aggctgagga tgttgggatt tattactgca tgcaaaatat acagcttccg    360

tggacgttcg gccaagggac caaggtggaa atcaaacgaa ctgtggctgc accatctgtc    420

ttcatcttcc cgccatctga tgagcagttg aaatctggaa ctgcctctgt tgtgtgcctg    480

ctgaataact tctatcccag agaggccaaa gtacagtgga aggtggataa cgccctccaa    540

tcgggtaact cccaggagag tgtcacagag caggacagca aggacagcac ctacagcctc    600

agcagcaccc tgacgctgag caaagcagac tacgagaaac acaaagtcta cgcctgcgaa    660

gtcacccatc agggcctgag ctcgcccgtc acaaagagct tcaacagggg agagtgttag    720

tga                                                                  723
```

<210> SEQ ID NO 20
<211> LENGTH: 239
<212> TYPE: PRT
<213> ORGANISM: Homo sapiens

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<400> SEQUENCE: 20

Met Arg Leu Pro Ala Gln Leu Leu Gly Leu Leu Met Leu Trp Ile Pro
1 5 10 15

Gly Ser Ser Ala Asp Ile Val Met Thr Gln Thr Pro Leu Ser Leu Ser
20 25 30

Val Thr Pro Gly Gln Pro Ala Ser Ile Ser Cys Lys Ser Ser Gln Ser
35 40 45

Leu Leu His Thr Asp Gly Thr Thr Tyr Leu Tyr Trp Tyr Leu Gln Lys
50 55 60

Pro Gly Gln Pro Pro Gln Leu Leu Ile Tyr Glu Val Ser Asn Arg Phe
65 70 75 80

Ser Gly Val Pro Asp Arg Phe Ser Gly Ser Gly Ser Gly Thr Asp Phe
85 90 95

Thr Leu Lys Ile Ser Arg Val Glu Ala Glu Asp Val Gly Ile Tyr Tyr
100 105 110

Cys Met Gln Asn Ile Gln Leu Pro Trp Thr Phe Gly Gln Gly Thr Lys
115 120 125

Val Glu Ile Lys Arg Thr Val Ala Ala Pro Ser Val Phe Ile Phe Pro
130 135 140

Pro Ser Asp Glu Gln Leu Lys Ser Gly Thr Ala Ser Val Val Cys Leu
145 150 155 160

Leu Asn Asn Phe Tyr Pro Arg Glu Ala Lys Val Gln Trp Lys Val Asp
165 170 175

Asn Ala Leu Gln Ser Gly Asn Ser Gln Glu Ser Val Thr Glu Gln Asp
180 185 190

Ser Lys Asp Ser Thr Tyr Ser Leu Ser Ser Thr Leu Thr Leu Ser Lys
195 200 205

Ala Asp Tyr Glu Lys His Lys Val Tyr Ala Cys Glu Val Thr His Gln
210 215 220

Gly Leu Ser Ser Pro Val Thr Lys Ser Phe Asn Arg Gly Glu Cys
225 230 235

<210> SEQ ID NO 21
<211> LENGTH: 543
<212> TYPE: PRT
<213> ORGANISM: Homo sapiens

<400> SEQUENCE: 21

Met Asp Phe Gly Leu Ala Leu Leu Leu Ala Gly Leu Leu Gly Leu Leu
1 5 10 15

Leu Gly Gln Ser Leu Gln Val Lys Pro Leu Gln Val Glu Pro Pro Glu
20 25 30

Pro Val Val Ala Val Ala Leu Gly Ala Ser Arg Gln Leu Thr Cys Arg
35 40 45

Leu Ala Cys Ala Asp Arg Gly Ala Ser Val Gln Trp Arg Gly Leu Asp
50 55 60

Thr Ser Leu Gly Ala Val Gln Ser Asp Thr Gly Arg Ser Val Leu Thr
65 70 75 80

Val Arg Asn Ala Ser Leu Ser Ala Ala Gly Thr Arg Val Cys Val Gly
85 90 95

Ser Cys Gly Gly Arg Thr Phe Gln His Thr Val Gln Leu Leu Val Tyr
100 105 110

-continued

```
Ala Phe Pro Asp Gln Leu Thr Val Ser Pro Ala Ala Leu Val Pro Gly
        115                 120                 125

Asp Pro Glu Val Ala Cys Thr Ala His Lys Val Thr Pro Val Asp Pro
    130                 135                 140

Asn Ala Leu Ser Phe Ser Leu Leu Val Gly Gly Gln Glu Leu Glu Gly
145                 150                 155                 160

Ala Gln Ala Leu Gly Pro Glu Val Gln Glu Glu Glu Glu Glu Pro Gln
                165                 170                 175

Gly Asp Glu Asp Val Leu Phe Arg Val Thr Glu Arg Trp Arg Leu Pro
            180                 185                 190

Pro Leu Gly Thr Pro Val Pro Pro Ala Leu Tyr Cys Gln Ala Thr Met
        195                 200                 205

Arg Leu Pro Gly Leu Glu Leu Ser His Arg Gln Ala Ile Pro Val Leu
    210                 215                 220

His Ser Pro Thr Ser Pro Glu Pro Pro Asp Thr Thr Ser Pro Glu Ser
225                 230                 235                 240

Pro Asp Thr Thr Ser Pro Glu Ser Pro Asp Thr Thr Ser Gln Glu Pro
                245                 250                 255

Pro Asp Thr Thr Ser Gln Glu Pro Pro Asp Thr Thr Ser Gln Glu Pro
            260                 265                 270

Pro Asp Thr Thr Ser Pro Glu Pro Pro Asp Lys Thr Ser Pro Glu Pro
        275                 280                 285

Ala Pro Gln Gln Gly Ser Thr His Thr Pro Arg Ser Pro Gly Ser Thr
    290                 295                 300

Arg Thr Arg Arg Pro Glu Ile Gln Pro Lys Ser Cys Asp Lys Thr His
305                 310                 315                 320

Thr Cys Pro Pro Cys Pro Ala Pro Glu Leu Leu Gly Gly Pro Ser Val
                325                 330                 335

Phe Leu Phe Pro Pro Lys Pro Lys Asp Thr Leu Met Ile Ser Arg Thr
            340                 345                 350

Pro Glu Val Thr Cys Val Val Val Asp Val Ser His Glu Asp Pro Glu
        355                 360                 365

Val Lys Phe Asn Trp Tyr Val Asp Gly Val Glu Val His Asn Ala Lys
    370                 375                 380

Thr Lys Pro Arg Glu Glu Gln Tyr Asn Ser Thr Tyr Arg Val Val Ser
385                 390                 395                 400

Val Leu Thr Val Leu His Gln Asp Trp Leu Asn Gly Lys Glu Tyr Lys
                405                 410                 415

Cys Lys Val Ser Asn Lys Ala Leu Pro Ala Pro Ile Glu Lys Thr Ile
            420                 425                 430

Ser Lys Ala Lys Gly Gln Pro Arg Glu Pro Gln Val Tyr Thr Leu Pro
        435                 440                 445

Pro Ser Arg Asp Glu Leu Thr Lys Asn Gln Val Ser Leu Thr Cys Leu
    450                 455                 460

Val Lys Gly Phe Tyr Pro Ser Asp Ile Ala Val Glu Trp Glu Ser Asn
465                 470                 475                 480

Gly Gln Pro Glu Asn Asn Tyr Lys Ala Thr Pro Pro Val Leu Asp Ser
                485                 490                 495
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-continued

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Asp Gly Ser Phe Phe Leu Tyr Ser Lys Leu Thr Val Asp Lys Ser Arg
            500                 505                 510

Trp Gln Gln Gly Asn Val Phe Ser Cys Ser Val Met His Glu Ala Leu
        515                 520                 525

His Asn His Tyr Thr Gln Lys Ser Leu Ser Leu Ser Pro Gly Lys
    530                 535                 540
```

What is claimed is:

1. A composition comprising:

at least one human IgG_2 antibody; and

a chelating agent.

2. The composition according to claim **1**, wherein the composition is a liquid pharmaceutical composition and the antibody does not comprise a signal sequence.

3. The composition according to claim **1**, wherein the composition comprises at least one chelating agent that is EDTA.

4. The composition according to claim **1**, wherein the composition further comprises a buffer.

5. The composition according to claim **1**, wherein the composition comprises at least one chelating agent that is EDTA, and further comprises at least one buffer that is histidine.

6. The composition according to claim **1**, wherein the composition further comprises a buffer and a surfactant.

7. The composition according to claim **1**, wherein the composition further comprises a buffer, a surfactant, and a tonicity agent.

8. The composition according to claim **1**, wherein the composition comprises at least one chelating agent that is EDTA, and further comprises a buffer, a surfactant, and a tonicity agent.

9. The composition according to claim **1**, wherein the composition comprises at least one chelating agent that is EDTA, and further comprises, histidine, a surfactant, and a tonicity agent.

10. The composition according to claim **1**, wherein the composition comprises at least one chelating agent that is EDTA, and further comprises histidine, polysorbate 80, and a tonicity agent.

11. The composition according to claim **1**, wherein the composition comprises:

from about 0.1 mg/ml to about 200 mg/ml of antibody;

from about 0.001 millimolar to about 5.0 millimolar of a chelating agent; and

from about 1 mM to about 100 mM of histidine.

12. The composition according to claim **1**, wherein the composition comprises:

from about 1 mg/ml to about 200 mg/ml of antibody;

from about 0.01 millimolar to about 5.0 millimolar of EDTA; and

from about 1 mM to about 100 mM of histidine.

13. The composition according to claim **1**, wherein the composition comprises:

from about 1 mg/ml to about 200 mg/ml of antibody;

from about 0.01 millimolar to about 1.0 millimolar of a chelating agent;

from about 1 mM to about 100 mM of a buffer;

from about 0.01 mg/ml to about 10 mg/ml of a surfactant; and

from about 100 millimolar to about 400 millimolar of a tonicity agent.

14. The composition according to claim **1**, wherein the composition comprises:

from about 1 mg/ml to about 200 mg/ml of antibody;

from about 0.01 millimolar to about 1.0 millimolar of EDTA;

from about 1 mM to about 100 mM of a buffer;

from about 0.01 mg/ml to about 10 mg/ml of a surfactant; and

from about 100 millimolar to about 400 millimolar of a tonicity agent.

15. The composition according to claim **1**, wherein the composition comprises:

from about 1 mg/ml to about 200 mg/ml of antibody;

from about 0.01 millimolar to about 1.0 millimolar of EDTA;

from about 1 mM to about 100 mM of histidine;

from about 0.01 mg/ml to about 10 mg/ml of a surfactant; and

from about 100 millimolar to about 400 millimolar of a tonicity agent.

16. The composition according to claim **1**, wherein the composition comprises:

from about 1 mg/ml to about 200 mg/ml of antibody;

from about 0.01 millimolar to about 1.0 millimolar of EDTA;

from about 1 mM to about 100 mM of histidine;

from about 0.01 mg/ml to about 10 mg/ml of polysorbate 80; and

from about 100 millimolar to about 400 millimolar of a tonicity agent.

17. The composition according to claim **1**, wherein the composition comprises:

from about 1 mg/ml to about 200 mg/ml of antibody;

from about 0.01 millimolar to about 1.0 millimolar of EDTA;

from about 1 mM to about 100 mM of histidine;

from about 0.01 mg/ml to about 10 mg/ml of polysorbate 80; and

from about 100 millimolar to about 400 millimolar of trehalose.

18. A composition comprising at least one monoclonal human IgG2 antibody and a chelating agent, wherein the composition comprises an amount of the chelating agent sufficient to stabilize the composition when maintained at a temperature of about 40° C. for a period of at least about 26 weeks.

19. A liquid pharmaceutical composition, comprising at least one human IgG2 monoclonal antibody and a pharmaceutically acceptable chelating agent, wherein the molar concentration of the antibody ranges from about 0.0006 millimolar to about 1.35 millimolar and the molar concentration of the chelating agent ranges from about 0.003 millimolar to

about 50 millimolar, and wherein the molar ratio of antibody to chelating agent ranges from about 0.00001 to about 2000.

20. The composition according to claim **19**, wherein the molar ratio of antibody to chelating agent ranges from about 0.01 to about 500.

21. The composition according to claim **19**, wherein the molar ratio of antibody to chelating agent ranges from about 0.05 to about 100.

22. The composition according to claim **19**, wherein the molar ratio of antibody to chelating agent ranges from about 0.1 to about 10.

23. The composition according to claim **19**, wherein the molar ratio of antibody to chelating agent ranges from about 0.5 to about 4.

24. The composition according to claim **19**, wherein the composition further comprises histidine in a molar concentration ranging from about 1 millimolar to about 100 millimolar.

25. A process for preparing a liquid human IgG2 pharmaceutical composition comprising mixing at least one human IgG2 antibody in solution; with at least one chelating agent.

* * * * *