

(54) T T O 20

TTO 20, DNA, , TTO 20 DNA TTO 20 ,

1

, TTO 20 , , , ,

TTO 20 DNA, TTO 20 (modulator)

- 1 (IL - 1) 가 . IL - 1 IL - 1 (PG) E₂

, - IL - 1, PG 가 , IL - 1 [Beuton HP & Ty

ler JA, 1988, Biochem. Biophys. Res. Comm. 154, 421 - 428; Aydelotte MB et al. Comm. Tiss. Res. 28, 143 - 159, Wood DD et al., Arthritis Rheum. 26, 975 - 983; Lohmander LS et al., Trans. Orthop. Res. Soc. 17, 273].

[EP 0 705 842 A2] RNA IL - 1 가

DDRT - PCR) RNA IL - 1 mRNA (

[Liang P & Gardee AB 1992, Science 257, 967 - 971; Liang P et al., AB 1993, Nucl. Acids Res. 21, 3269 - 3275; Bauer D et al. 1993, Nucl. Acids Res. 21, 4272 - 4280].

mRNA mRNA (decamer)

mRNA , DNA , 100 , 10000 PCR

가

1. DDRT - PCR

2.

3. RNA

[EP 0 705 842 A2] , DNA

cDNA : , TNF - 100% 6(TSG - 6) cDNA 99.5% , 97.2% 가

cDNA 400bp - TTO 20/2(2) , DNA 152bp

TTO 20/2

가

TTO 20

mRNA

RNA TTO 20/2
RNA가

pED4

[Kaufmann et al., 1991, Nucl. Acids Res. 19, 4485 - 4490]

RNA) , mRNA(RNA) , RNA(RNA)

mRNA 3' 5'

EST

" - " EST

mRNA

가

가

가 , RNA

DNA 가 - 가 , EST가 가 , HTS DNA

EST pED4 96 - 3' PST HTS 5' EcoRI DNA

PCR PCR 2 , PCR PCR

PstI EcoRI EGFP : Boehringer, Mannheim), (: Life Tech

CaPO₄, Fugene 6(nologies, Eggenstein)

CO₂ , 24 72 가 PstI - EcoRI 가 DNA

가 RNA 가 DNA

가 EST 가 가

1 TTO 20

가 , (a) 1 DNA , 1 1305 ; (b) (a) DNA 80% DNA (c) , (a) (b) DNA DNA

가 TTO 20

가 DNA

, (a) TTO 20 (b) TTO 20

가 , TTO 20 DNA RNA

TTO 20 / PCR 1 DNA 1

TO 20 가 TTO 20 3 가 TTO 20 TTO 20 T

가 , DNA ,

1 : TTO 20/2 cDNA

TTO 20/2 152bp [EP 0 705 842 A2]
 , TTO 20/2 DNA SP6
 [Sanger, F. et al. 1977, Proc. Natl. Acad. Sci. USA 74, 5463 - 5467] DNA
 , 272bp 가
 (NCBI) EMBL , BLAST FASTA
 (Genetics Computer Group, Madison, USA) GCG DNA

5077(ID: g868629) EST M 51303(ID: g11924769) H 0
 (Genome Systems, St. Louis, USA) ID 283071 43508 ID 283
 071 2580bp 가 가 , ID 43508 2200bp
 , 283071 100% 283071 1
 706

2 :

GCG (Translate program) , 283071 DNA
 (ORF) , 200 5'
 TAA , 1950bp
 A 1 706 3262

5' DNA
 : g87458) cDNA 184 , KIAA0282(
 G 552bp KIAA0282 LQTSE
 TAA 283071 15

, KIAA0282 ORF 123 , KIAA0282 2000bp
 3' 283071 , 1

3 : KIAA0282 5' TTO 20 3'

KIAA0282 ORF 5' TTO 20 3' 가
 EST 43508 , 47831 36563
 (Genome Systems) TTO 20

KIAA 5' TTO 20 3' , PC
 R KIAA0282 5' 1: 5' - CAACTCC
 TGTAT CACC - 3' (1), 2: 5' - AGTGCGATGTCTTCTACTG - 3' (2). 3 4
 283071 3' 3: 5' - TCTAAAGGCAG GAGAGGAAC - 3' (3).
 4: 5' - TTCATGTGTCTTGCTTACTC - 3' (4). , 2097bp 1 4
 , 1211bp 2 3 DNA
 : Multiple Tissue cDNA Panel Human 1(: K1420 - 1,
 : Clontech, Heidelberg, Germany) cDNA Human Fetal cDNA Panel(: K1425 - 1,
 : Clontech, Heidelberg, Germany) cDNA. 0.5 1μℓ 1 4 PC
 R . PCR :
 : AmpliTag DNA (: Perkin - Elmer, Weiterstadt, Germany) 19 pfu ((: Stratagen, La Jolla, USA) 1 . : 10pM, DNA (: Perkin Elmer, Model 480). 1
 PCR : 94 2 , 94 30 , 59 30 , 72 30 , 72 7 , : 50μℓ. 25 30
 , 4 1.2% 가 . cDNA
 cDNA 2100bp PCR 가 . 0.5
 1μℓ , 2 3 가 , (nested)" PCR , 1
 200bp PCR KIAA0282 5' TTO 20 3' cDNA

가 , 2100bp DNA 가 DNA
 [Ausubel et al.]. DNA 10μℓ (Invitrogen BV, Groningen, Holland)
 pCR2.1 . PCR
 TA
 DNA 377 (: Perkin Elmer, Langen, German
 y) [Sanger et al.] PCR
 DNA 5' , 1 (685 3262) KIAA0282
 1 (1 3262).

4 :

EST . RNA [: EP - A 0 705 842] 1 - 4
 , KIAA0282 cDNA (zinc finger)
 . TTO 20 가 , (1
 705 3198) . cDNA
 3' .

4.1

pED4 (2)[Kaufman et al., Nucleic Acids Research 19, 448 - 4490
 (1991)]. [Kaufman et al.] pMT2 A
 TCC ATCC 67122 , RNA 1
 :
 SV40 , , mRNA ,

(encephalomyocarditis)
 , SV40 , V1 RNA ,
 rescent protein, GFP) . 1 , (green fluo
 가 , 2 GFP

pED4 DNA Clal XhoI GFP
 pEGFP(: Clontech, Heidelberg, Germany) DNA Sall NotI
 . DNA . Clal 가 VA
 350bp , 3 A VA
 . PCR , 5' Not 가 3' Cla 가 , A
 VA pED4 DNA .

Not L:

5' - ATAAGAATGCGGCCGCTAAACTATCAGGAAGATGCTTTCAAGTTC - 3' (5)

Cla R:

5' - ACAGGCTCTCCTTTTGCAC - 3' (6).

PCR NotI Clal , ATP T4 가 . DH5
 DNA BamHI pED4 BamHI 가 ,
 2950bp, 2190bp 220bp DNA
 T782 BamHI 가 DNA BamHI , 2950bp, 13
 15bp, 1075bp 220bp DNA
 T782 3 .

TTO 20 RNA GFP T782 , TTO 20 PCR EcoRI

5' - GGAATTCGATCAGATCTCTCACTGCAC - 3' (7)

5' - GGAATTCACCTTCTGTGCAGTAACAGAG - 3' (8)

2500bp
 EcoRI EcoRI
 가 가
 GFP
 DH5
 PstI 가
 DNA 310bp Pst
 810bp
 T814 Pst 4

4.2

가 가
 MOD (4.5g/ DMEM) 가 (10%
 , 200mM L - , 5mM , 20mM Hepes , pH 7.2, 0.02µg
 /Ml, 0.1µg/Ml, 0.025mg/Ml 0.05µg/Ml) (75cm²)
 .3 4 , 10Ml , /EDTA
 0.25% 1mM EDTA 1:5 1:10 1Ml 37 2 5
 10% DMSO 90% - 80

DNA , DEAE -
 (dendrimer)
 가 Fugene 6(: Boehringer Mannheim, Mannheim, Germany)
 2 x 10⁵ 4 x 10⁵ 6 (: Beckton & Dickinson, Heidelberg, Germany)
 1 2Ml (Qiafilter Plasmid Mi
 dikit, : Qiagen, Hilden, Germany) DNA 0.5 2µg
 5% CO₂, 37 18 24 5
 0 80% 4 x 10⁵ / , DNA 2
 µg Fugene 6µl/ 100µl

4.3

GFP
 16 48 .16 , 20
 가 RNA , 6
 . RNA (RNeasy Miniprep Kit, : Qiagen, Hilden,
 Germany) . RNA RNase - 30µl - 20
 RNA - DNA [Ausubel, F.M. et al. Current Protocols in Molecul
 arbiology, Vol. 1 - 3, John Wiley and Sons, New York, 1997] . DNA DIG
 (DIG Probe Synthesis Kit, : Boehringer, Mannheim, Germany) PCR
 2.5kb TTO 20 P
 CR . RNA
 - 가 .

O 20 - , (cofilin) RNA , TT
 , GFP 가 가 TTO 20
 RNA .

1 283071, 43508, KIAA0282, TTO 20/2 g 868629 .

2 pED4 , : SV40 = SV40 ; MLP =
 " " ; TPL = " " mRNA; IVS =
 ; EM C - L = ; DHFR = ; p
 olyA = SV40 ; VA1 = VA I RNA .

3 T782 , 2 .

4 T814 , 2 .

1번 내지 3262번의 T10 20-2 유전자

1 GCCCTGGCCCCGGTGCCCGCAACTCCTGTATCACCTGCCCCCAGTGTACCCGCAGCCTC
 1 A L A P V P R N S C I T C P Q C H R S L -
 61 ATCCTGGATGACCGGGGGCTCCGCGGCTTCCCCAAGAATCGCGTACTGGAAGGGGTAATT
 21 I L D D R G L R G F P K N R V L E G V I -
 121 GACCGCTACCAGCAGAGCAAAGCCGCGGCCCTCAAGTGCCAGCTCTGCGAGAAGGCGCCC
 41 D R Y Q Q S K A A A L K C Q L C E K A P -
 181 AAGGAAGCCACCGTCATGTGCGAACAGTGCGATGTCTTCTACTGCGATCCGTGCCGCCTG
 61 K E A T V M C E Q C D V F Y C D P C R L -
 241 CGCTGCCACCCGCCCCGGGGGCCCTAGCCAAGCACCGCCTGGTGCCCCCGGCCAGGGT
 81 R C H P P R G P L A K H R L V P P A Q G -
 301 CGTGTGAGCCGGAGGCTGAGCCCACGCAAGGTCTCCACCTGCACAGACCACGAGCTGGAG
 101 R V S R R L S P R K V S T C T D H E L E -
 361 AACCACAGCATGTACTGCGTGCAATGCAAGATGCCCGTGTGCTACCAGTGCTTGGAGGAG
 121 N H S M Y C V Q C K M P V C Y Q C L E E -
 421 GGCAAACACTCCAGCCACGAAGTCAAGGCTCTGGGGGCCATGTGGAAACTACATAAGAGC
 141 G K H S S H E V K A L G A M W K L H K S -
 481 CAGCTCTCCAGGCGCTGAACGGACTGTCAGACAGGGCCAAAGAAGCCAAGGAGTTTCTG
 161 Q L S Q A L N G L S D R A K E A K E F L -
 541 GTACAGCTGCGCAACATGGTCCAGCAGATCCAGGAGAACAGTGTGGAGTTTGAAGCCTGT
 181 V Q L R N M V Q Q I Q E N S V E F E A C -
 601 CTGGTGGCCCAATGTGATGCCCTCATCGATGCCCTCAACAGAAGAAAAGCCCAGCTGCTG
 201 L V A Q C D A L I D A L N R R K A Q L L -
 661 GCCCGCGTCAACAAGGAGCATGAGCACAAGCTGAAGGTGGTTCGAGATCAGATCTCTCAC
 221 A R V N K E H E H K L K V V R D Q I S H -
 721 TGCACAGTGAAATTGCGCCAGACCACAGGTCTCATGGAGTACTGCTTGGAGGTGATTAAG
 241 C T V K L R Q T T G L M E Y C L E V I K -
 781 GAAAATGATCCTAGTGGTTTTTTGCAGATTTCTGACGCCCTCATAAGAAGAGTGCACCTG
 261 E N D P S G F L Q I S D A L I R R V H L -

841 ACTGAGGATCAGTGGGGTAAAGGCACACTCACTCCAAGGATGACCACGGACTTTGACTTG
 281 T E D Q W G K G T L T P R M T T D F D L -
 901 AGTCTGGACAACAGCCCTCTGCTGCAATCCATCCACCAGCTGGATTTCTGTGCAAGTGAAA
 301 S L D N S P L L Q S I H Q L D F V Q V K -
 961 GCTTCCTCTCCAGTCCCAGCAACCCCTATCCTACAGCTGGAGGAATGTTGTACCCACAAC
 321 A S S P V P A T P I L Q L E E C C T H N -
 1021 AACAGCGCTACGTTGTCTGGAAACAGCCACCTCTGTCCACGGTGCCCGCCGATGGATAC
 341 N S A T L S W K Q P P L S T V P A D G Y -
 1081 ATTCTGGAGCTGGATGATGGCAACGGTGGTCAATTCCGGGAGGTGTATGTGGGGAAGGAG
 361 I L E L D D G N G G Q F R E V Y V G K E -
 1141 ACAATGTGCACTGTGGATGGTCTTCACTTCAACAGCACATACAACGCTCGGGTCAAGGCC
 381 T M C T V D G L H F N S T Y N A R V K A -
 1201 TTCAACAAAACAGGAGTCAGCCCGTACAGCAAGACCCTGGTCCTCCAAACGTCTGAGGGT
 401 F N K T G V S P Y S K T L V L Q T S E G -
 1261 AAGGCCCTTCAGCAGTATCCCTCAGAGCGAGAACTGCGTGGCATCTAAAGTGGCTGGCAA
 421 K A L Q Q Y P S E R E L R G I * (서열 10)
 1321 GCCCGGAGGTAACCCACCCTGCCCACATTCCTGAAGTGTTCATGACTTGCTCTGCA
 1381 TTCTGCACAGAGCCGCTGTTCTCTCCTGCCTTTAGAGAGCCTATGGTATGTGGATGTGA
 1441 TCAAACCAAAGATTCCACATCGGCAGTTCCAATGGCTTGGGCCGGCGGCTTCCTTTGATA
 1501 ACAATCTAAATAAGCTGCAGTTGAAGAAGCTGAAAAATGAAGGCCTGAATGTGCCCTGG
 1561 TGTGTAAGACAAATGTATCTAGGCTCTAGAGCAGGCTCCCATTCCTCCACCGATACACATC
 1621 ATGTGCCAGTTTTGCCAGATGATTCATAAATACTCTGTAGTACTTGCTTGTTCTGAGGG
 1681 TGGGACCTAGGTTCTTCCAGTCGTGGATTTGTATGACTGAATGTGTTTCAAATGGGTGG
 1741 TGGGGTGCTAGAGCTGTTTAGAGAGGGCCTGTTGGCTGCTCCTGGCTTACCCACTTAGAC
 1801 TGCTCCGTTTCATACCCAAAGCGGAGGCCGTCAGCACCAGGATTGAGACTTCCTGTGGG
 1861 CACCAAACAGGAAGAGACCAGCAACTTCGCATTACCCGCCATTTTCATCTTTGCCAGTCC
 1921 CTTCACTCTGGCTAGGCTACCGAGAGCCACCATAAAGGTTTCATCTCTAGAGAATTTTT
 1981 GCTTCTTAGCTATACTTTAAATATTTGGTCATCAAAGACAAGTAATGTGTCTCAGATGA
 2041 GAGGCTGAATTTGATGGCCAGATATAACCTCTGAGGCTTTTAACATTTTCATTTAAGA
 2101 GTAAGCAAGACACATGAAATTAACCTACAAGGAGGTATTTGTGGCTGGTGCCAAAGCAT
 2161 TCTGACACTTTGGGGTGTCAATTTAATCAAAGAAATCACCCCCCACCTCACCGGGATTC

2221 TCCATAACTTCCCTCTGCAGAACTAATTATGTTGATTTTGTTC AAGTTCAAGATGTTAGC
 2281 TAAAAGAACTATGGTGGTGTTTTTTTTCCCACTTCCCACAAACCTCAACATGTGCCAGTC
 2341 ACCCTAAAATGCTTCACATGGTTAAAAACAAGCACAATTTTGAATCCTACAGCAAGGATG
 2401 AAAGGCCCTAGCCGTGAAAACAGTGCTTTGGGGAGCAGTTGTCAGTCTAAGTGATGCTA
 2461 TTCCAAGAGAAGGATATGCTGAGGGAAAATGCCACATGACCTGTTCCCATTGGAGTAC
 2521 AGTGATGTGGTAGCTACAGCTTCCCCAGAATTATCATTTTAGCACCTTCTCTCAGGGAT
 2581 GACCTATCAGTTTGAGAGCAGTTGCCTCTTTTCTCAA AATACCATACTAACTGCTAAAGC
 2641 CCTCCAAGAGTCTTTCCTAGATGCAACGCAGAAGGCCCTTGCTGGTGATGGCCTCATTTT
 2701 CCATGTGTGTACAAGGTGGTTTGATTGAAGAGTGAAGTGCATGCCTGCAGAGCAGAGAGA
 2761 AATTTGTAGCAATGTTGCTAATATGTGTTATCAGATCTGCGGGAAA ACTATTTCTATTCA
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 2881 GCACTTATGTCTCTCGTTTCATCCTGCAGGAGGATGTAGCGCCTCAGTTTATTTTAATGT
 2941 TCATAAGATTATGGTGTCTAATTTAATAAATTACAGGATTGGAACTGCGATCCTTGGTAC
 3001 CACAGTCACAGA ACTGGGGTCA TTTTCTAGATGAAACAAACGGAACAAGTTCTCTTCCA
 3061 ACAAAGAACTGTACTGTAGAAATTAATTTCTCCATGAATTTTATATATTGTGTACAAA
 3121 TATAAGGTATGTATCTGAATACAAAGAAAAGCCTATCATCATATAGATATCAGTATTCTC
 3181 TGTTACTGCACAGAAGTAATTTTCTCATGATGAAATAAAGTTTACACACATACTTTCTCC
 3241 ATAAAAAAAAAAAAAAAAAAAAA 3262 (서열 9)

(57)

1.

1 (10) TTO 20 .

2.

(a) 1 DNA (9), 1 1305 ; (b) (a) DNA 80% DNA;
 (c) , (a) (b) DNA DNA
 1 DNA.

3.

1

4.

2 DNA

5.

4

6.

(a) TTO 20 가 , 1 TTO 20 ; (b)

7.

1 , 6 TTO 20

8.

2 DNA RNA , 1 TTO 20

9.

/ PCR 1 DNA 2 (a) DNA

10.

TTO 20 3 1 TTO 20

11.

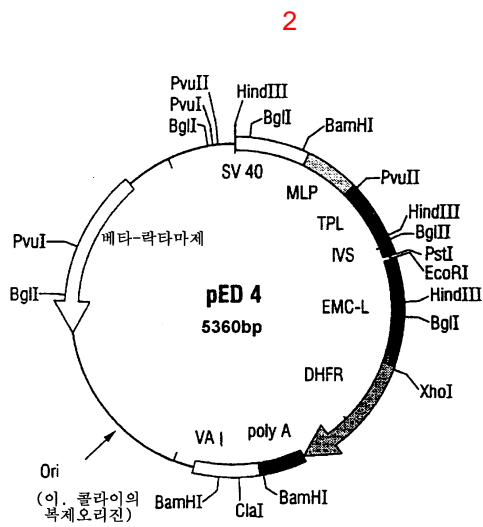
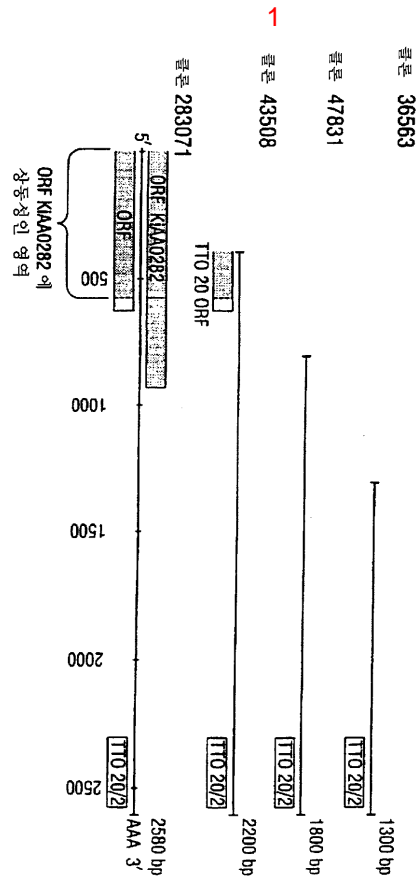
TTO 20 1 TTO 20

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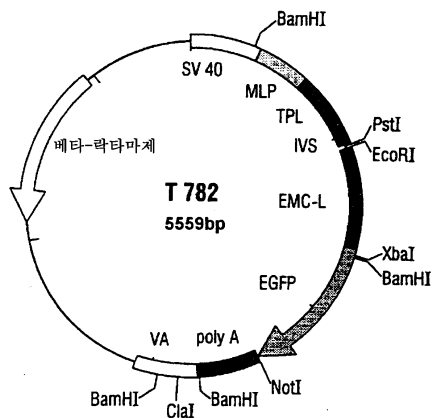
3 , ,

13.

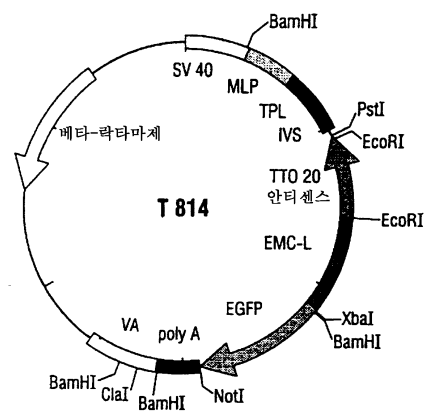
2 DNA , ,



3



4



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 35 40 45
 Ala Ala Leu Lys Cys Gln Leu Cys Glu Lys Ala Pro Lys Glu Ala Thr
 50 55 60
 Val Met Cys Glu Gln Cys Asp Val Phe Tyr Cys Asp Pro Cys Arg Leu
 65 70 75 80
 Arg Cys His Pro Pro Arg Gly Pro Leu Ala Lys His Arg Leu Val Pro
 85 90 95
 Pro Ala Gln Gly Arg Val Ser Arg Arg Leu Ser Pro Arg Lys Val Ser
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 Thr Cys Thr Asp His Glu Leu Glu Asn His Ser Met Tyr Cys Val Gln
 115 120 125
 Cys Lys Met Pro Val Cys Tyr Gln Cys Leu Glu Glu Gly Lys His Ser
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 Ser His Glu Val Lys Ala Leu Gly Ala Met Trp Lys Leu His Lys Ser
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 Lys Glu Phe Leu Val Gln Leu Arg Asn Met Val Gln Gln Ile Gln Glu
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 Asn Ser Val Glu Phe Glu Ala Cys Leu Val Ala Gln Cys Asp Ala Leu
 195 200 205
 Ile Asp Ala Leu Asn Arg Arg Lys Ala Gln Leu Leu Ala Arg Val Asn
 210 215 220
 Lys Glu His Glu His Lys Leu Lys Val Val Arg Asp Gln Ile Ser His
 225 230 235 240
 Cys Thr Val Lys Leu Arg Gln Thr Thr Gly Leu Met Glu Tyr Cys Leu
 245 250 255
 Glu Val Ile Lys Glu Asn Asp Pro Ser Gly Phe Leu Gln Ile Ser Asp
 260 265 270
 Ala Leu Ile Arg Arg Val His Leu Thr Glu Asp Gln Trp Gly Lys Gly
 275 280 285
 Thr Leu Thr Pro Arg Met Thr Thr Asp Phe Asp Leu Ser Leu Asp Asn
 290 295 300
 Ser Pro Leu Leu Gln Ser Ile His Gln Leu Asp Phe Val Gln Val Lys
 305 310 315 320

