

(43) International Publication Date
13 January 2011 (13.01.2011)(10) International Publication Number
WO 2011/005773 A3

PCT

(51) International Patent Classification:
C12M 3/02 (2006.01) *C12M 1/00* (2006.01)(21) International Application Number:
PCT/US2010/041082(22) International Filing Date:
6 July 2010 (06.07.2010)

(25) Filing Language: English

(26) Publication Language: English

(30) Priority Data:
61/223,313 6 July 2009 (06.07.2009) US(71) Applicant (for all designated States except AL, AT, BA, BE, BG, CH, CN, CY, CZ, DE, DK, EE, ES, FI, FR, GB, GR, HR, HU, IE, IN, IS, IT, LT, LU, LV, MC, MK, MT, NL, NO, PL, PT, RO, RS, SE, SI, SK, SM, TR, US):
GENENTECH, INC. [US/US]; 1 DNA Way, South San Francisco, CA 94080 (US).(71) Applicant (for AL, AT, BA, BE, BG, CH, CN, CY, CZ, DE, DK, EE, ES, FI, FR, GB, GR, HR, HU, IE, IN, IS, IT, LT, LU, LV, MC, MK, MT, NL, NO, PL, PT, RO, RS, SE, SI, SK, SM, TR only): **F. HOFFMANN-LA ROCHE AG** [CH/CH]; Grenzacher Strasse 124, CH-4070 Basel (CH).

(72) Inventors; and

(75) Inventors/Applicants (for US only): **BASKAR, Dinesh** [IN/US]; 123 N. El Camino Real, #20, San Mateo, CA 94401 (US). **HSIUNG, Jenny** [US/US]; 137 Centre Street, Mountain View, CA 94041 (US). **LEUNG, Woon-Lam, Susan** [US/US]; 609 Cuesta Avenue, San Mateo, CA 94403 (US). **YUK, Inn, H.** [US/US]; 1729 Sixth Street, Berkeley, CA 94710 (US).(74) Agents: **FARLEY, Patrick, J.** et al.; Genentech, Inc., 1 DNA Way, MS-49, South San Francisco, CA 94080 (US).

(81) Designated States (unless otherwise indicated, for every kind of national protection available): AE, AG, AL, AM, AO, AT, AU, AZ, BA, BB, BG, BH, BR, BW, BY, BZ, CA, CH, CL, CN, CO, CR, CU, CZ, DE, DK, DM, DO, DZ, EC, EE, EG, ES, FI, GB, GD, GE, GH, GM, GT, HN, HR, HU, ID, IL, IN, IS, JP, KE, KG, KM, KN, KP, KR, KZ, LA, LC, LK, LR, LS, LT, LU, LY, MA, MD, ME, MG, MK, MN, MW, MX, MY, MZ, NA, NG, NI, NO, NZ, OM, PE, PG, PH, PL, PT, RO, RS, RU, SC, SD, SE, SG, SK, SL, SM, ST, SV, SY, TH, TJ, TM, TN, TR, TT, TZ, UA, UG, US, UZ, VC, VN, ZA, ZM, ZW.

(84) Designated States (unless otherwise indicated, for every kind of regional protection available): ARIPO (BW, GH, GM, KE, LR, LS, MW, MZ, NA, SD, SL, SZ, TZ, UG, ZM, ZW), Eurasian (AM, AZ, BY, KG, KZ, MD, RU, TJ, TM), European (AL, AT, BE, BG, CH, CY, CZ, DE, DK, EE, ES, FI, FR, GB, GR, HR, HU, IE, IS, IT, LT, LU, LV, MC, MK, MT, NL, NO, PL, PT, RO, SE, SI, SK, SM, TR), OAPI (BF, BJ, CF, CG, CI, CM, GA, GN, GQ, GW, ML, MR, NE, SN, TD, TG).

Published:

- with international search report (Art. 21(3))
- before the expiration of the time limit for amending the claims and to be republished in the event of receipt of amendments (Rule 48.2(h))

(88) Date of publication of the international search report:
26 January 2012

(54) Title: METHOD OF CULTURING EUKARYOTIC CELLS

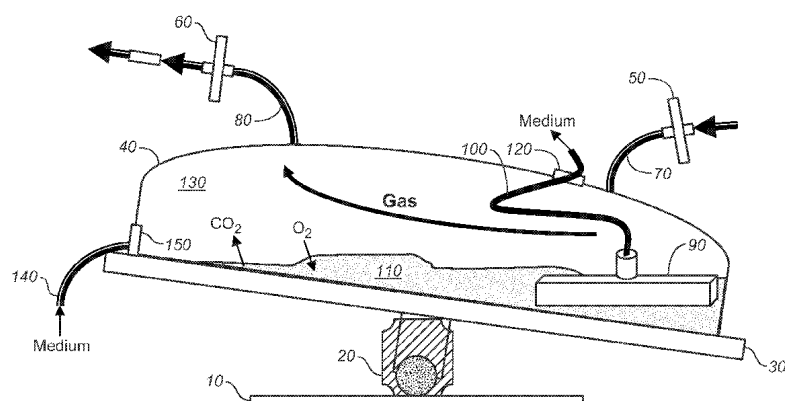


FIG. 1

(57) Abstract: An apparatus and method to maintain pH within a range conducive for cell growth in a bicarbonate-containing cell culture system without the addition of base. The method relies on the gas transfer characteristics of the bioreactor system to modulate the CO₂ transfer to and from the cell culture such that the pH of the cell culture can be maintained within a desired range.

WO 2011/005773 A3

A. CLASSIFICATION OF SUBJECT MATTER

INV. C12M1/00 C12M3/02

ADD.

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)

C12M

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practical, search terms used)

EP0-Internal, WPI Data

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	US 6 190 913 B1 (SINGH VIJAY [US]) 20 February 2001 (2001-02-20) column 1, lines 23-25 column 3, lines 44-67 column 4, lines 1-3,38-43,57-59 column 5, lines 17-20 pages -; claims	1-11, 33-42
X	----- EP 0 147 975 A2 (AJINOMOTO KK [JP]) 10 July 1985 (1985-07-10) pages 1-3,6-8 claims; figures	1-11, 33-42
X	----- DE 36 06 449 A1 (NEW BRUNSWICK SCIENTIFIC CO [US]) 4 September 1986 (1986-09-04) pages 8,10-12 claims; figures	33,35-38
	----- -/-	

☒ Further documents are listed in the continuation of Box C.☒ See patent family annex.

* Special categories of cited documents :

"A" document defining the general state of the art which is not considered to be of particular relevance

"E" earlier document but published on or after the international filing date

"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)

"O" document referring to an oral disclosure, use, exhibition or other means

"P" document published prior to the international filing date but later than the priority date claimed

"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone

"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art.

"&" document member of the same patent family

Date of the actual completion of the international search

6 December 2011

Date of mailing of the international search report

14/12/2011

Name and mailing address of the ISA/

European Patent Office, P.B. 5818 Patentlaan 2
NL - 2280 HV Rijswijk
Tel. (+31-70) 340-2040,
Fax: (+31-70) 340-3016

Authorized officer

Böhm, Ingo

INTERNATIONAL SEARCH REPORT

International application No.
PCT/US2010/041082

Box No. II Observations where certain claims were found unsearchable (Continuation of item 2 of first sheet)

This international search report has not been established in respect of certain claims under Article 17(2)(a) for the following reasons:

1. ☐ Claims Nos.:
because they relate to subject matter not required to be searched by this Authority, namely:

2. ☐ Claims Nos.:
because they relate to parts of the international application that do not comply with the prescribed requirements to such an extent that no meaningful international search can be carried out, specifically:

3. ☐ Claims Nos.:
because they are dependent claims and are not drafted in accordance with the second and third sentences of Rule 6.4(a).

Box No. III Observations where unity of invention is lacking (Continuation of item 3 of first sheet)

This International Searching Authority found multiple inventions in this international application, as follows:

see additional sheet

1. ☒ As all required additional search fees were timely paid by the applicant, this international search report covers all searchable claims.
2. ☐ As all searchable claims could be searched without effort justifying an additional fees, this Authority did not invite payment of additional fees.
3. ☐ As only some of the required additional search fees were timely paid by the applicant, this international search report covers only those claims for which fees were paid, specifically claims Nos.:
4. ☐ No required additional search fees were timely paid by the applicant. Consequently, this international search report is restricted to the invention first mentioned in the claims; it is covered by claims Nos.:

Remark on Protest

- ☐ The additional search fees were accompanied by the applicant's protest and, where applicable, the payment of a protest fee.
- ☐ The additional search fees were accompanied by the applicant's protest but the applicable protest fee was not paid within the time limit specified in the invitation.
- ☒ No protest accompanied the payment of additional search fees.

C(Continuation). DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	EP 2 020 433 A2 (XCELLEREX INC [US]) 4 February 2009 (2009-02-04) paragraphs [0001], [0009], [0010], [0038], [0048], [0054], [0107], [0126], [0127] claims; figures -----	33,35-38
X	EP 0 200 226 A1 (ROSIN HARRY PROF DR [DE]; PREISENDANZ HANS [DE]) 5 November 1986 (1986-11-05) claims; figures -----	1-43
X	WO 2005/118771 A2 (XCELLEREX INC [US]; HODGE GEOFFREY [US]; GALLIHER PARRISH [US]; FISHER) 15 December 2005 (2005-12-15) page 3, lines 22-31 page 6, lines 1-6,15-22 page 7, lines 15-20 pages 1,4,5,7; claims; figures pages 9,11,12 pages 20,22 claims; figures -----	33-38
A	US 2003/036192 A1 (SINGH VIJAY [US]) 20 February 2003 (2003-02-20) paragraphs [0008], [0009], [0020], [0023], [0026] claims; figures; table 1 -----	1-43
A	US 3 941 662 A (MUNDER PAUL GERHARD ET AL) 2 March 1976 (1976-03-02) column 4 claims; figures -----	1,12,33, 39,43

INTERNATIONAL SEARCH REPORT

Information on patent family members

International application No

PCT/US2010/041082

Patent document cited in search report	Publication date	Patent family member(s)	Publication date
US 6190913	B1	20-02-2001	NONE
EP 0147975	A2	10-07-1985	EP 0147975 A2 10-07-1985 JP 60141286 A 26-07-1985
DE 3606449	A1	04-09-1986	CH 669607 A5 31-03-1989 DE 3606449 A1 04-09-1986 FR 2578266 A1 05-09-1986 GB 2171818 A 03-09-1986 JP 2056399 C 23-05-1996 JP 7079678 B 30-08-1995 JP 61202684 A 08-09-1986 US 4680267 A 14-07-1987
EP 2020433	A2	04-02-2009	EP 2020433 A2 04-02-2009 JP 2009072182 A 09-04-2009 US 2009035856 A1 05-02-2009
EP 0200226	A1	05-11-1986	DE 3515753 A1 06-11-1986 EP 0200226 A1 05-11-1986
WO 2005118771	A2	15-12-2005	AU 2005250500 A1 15-12-2005 BR PI0510648 A 20-11-2007 CA 2569405 A1 15-12-2005 CR 8769 A 28-08-2007 EP 1773976 A2 18-04-2007 JP 4680990 B2 11-05-2011 JP 2008501347 A 24-01-2008 KR 20070053200 A 23-05-2007 WO 2005118771 A2 15-12-2005
US 2003036192	A1	20-02-2003	NONE
US 3941662	A	02-03-1976	NONE

FURTHER INFORMATION CONTINUED FROM PCT/ISA/ 210

This International Searching Authority found multiple (groups of) inventions in this international application, as follows:

1. claims: 1-11, 33-42

i) Claims 1-11

A method for batch culturing eukaryote cells comprising: providing cell culture inoculant comprising eukaryotic cells in a bicarbonate- containing culture liquid to a vessel, said vessel having walls that encapsulate said cell culture and a gas phase head space above said cell culture, and wherein said vessel comprises at least one port that provides an entrance and an egress of gas to and from said head space; agitating said vessel; and providing gas to said head space through said port wherein said gas contains an amount of CO₂, and wherein said amount of CO₂.

ii) Claims 33-38)

An apparatus for culturing eukaryote cells comprising: a vessel having walls define an interior space of the vessel and separate said interior space from a surrounding environment; at least one port that provides an entrance and an egress of gas from said vessel; an agitator reversibly attached to said vessel; and optionally, a pH monitor having a probe positioned in said interior space. 34. The apparatus of claim 33 wherein said vessel is a disposable bioreactor bag.

iii) Claims 39-42

A method for batch culturing eukaryote cells comprising: providing cell culture comprising eukaryotic cells in a bicarbonate- containing culture liquid to a vessel, said vessel having walls that encapsulate said cell culture and a gas phase head space above said cell culture, and wherein said vessel comprises at least one port that provides an entrance and an egress of gas to and from said head space; agitating said vessel; and providing gas to said head space through said port wherein said gas contains 8% CO₂ (v/v) of gas during the first day, 5% CO₂ (v/v) of gas the second day and 2% CO₂ (v/v) of gas thereafter.

2. claims: 12-32

i) Claim 12-32

A method for perfusion culturing eukaryote cells comprising: providing cell culture inoculant comprising eukaryotic cells in a bicarbonate- containing culture liquid to a vessel, said vessel having walls that encapsulate said cell culture and a gas phase head space above said cell culture, and wherein said vessel comprises at least one port that provides an entrance and an egress of gas to and from said

FURTHER INFORMATION CONTINUED FROM PCT/ISA/ 210

head space;
agitating said vessel;
perfusing fresh culture medium into said vessel and removing spent culture medium from said vessel; and
providing gas to said head space through said port to sweep accumulated CO₂ from the headspace of said vessel, thereby modulating the pH of said cell culture in order to maintain a predetermined pH of said cell culture.

3. claim: 43

i) Claim 43

A method for perfusion culturing eukaryote cells comprising:
providing cell culture comprising eukaryotic cells in a bicarbonate- containing culture liquid to a vessel, said vessel having walls that encapsulate said cell culture and a gas phase head space above said cell culture, and wherein said vessel comprises at least one port that provides an entrance and an egress of gas to and from said head space;
agitating said vessel;
providing gas to said head space through said port wherein said gas is supplemented with at least 30% O₂ (v/v) two days after initiation of perfusion.
