



INTERNATIONAL APPLICATION PUBLISHED UNDER THE PATENT COOPERATION TREATY (PCT)

(51) International Patent Classification ⁶ : C12N 7/00, C12P 21/00, C12N 5/00, A61K 39/145, C07K 14/11, A61K 39/15, 39/245, A61K 39/12	A3	(11) International Publication Number: WO 96/15231 (43) International Publication Date: 23 May 1996 (23.05.96)
(21) International Application Number: PCT/EP95/04439 (22) International Filing Date: 10 November 1995 (10.11.95) (30) Priority Data: 08/338,761 10 November 1994 (10.11.94) US 08/487,046 7 June 1995 (07.06.95) US 08/487,222 7 June 1995 (07.06.95) US 08/483,522 7 June 1995 (07.06.95) US (71) Applicant (for all designated States except US): IMMUNO AKTIENGESELLSCHAFT [AT/AT]; Industriestrasse 67, A-1221 Vienna (AT). (72) Inventors; and (75) Inventors/Applicants (for US only): KISTNER, Otfried [DE/AT]; Viktorgasse 17/6, A-1040 Vienna (AT). BARRETT, Noel [IE/AT]; Steinwandgasse 6A, A-3400 Klosterneuburg (AT). MUNDT, Wolfgang [AT/AT]; Florianigasse 57/1/2/6, A-1080 Vienna (AT). DORNER, Friedrich [AT/AT]; Peterlinigasse 17, A-1230 Vienna (AT). (74) Agents: KOLB, Helga et al.; Hoffmann, Eitle & Partner, Arabellastrasse 4, D-81925 Munich (DE).		(81) Designated States: CA, FI, JP, NO, US, European patent (AT, BE, CH, DE, DK, ES, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE). Published <i>With international search report.</i> <i>Before the expiration of the time limit for amending the claims and to be republished in the event of the receipt of amendments.</i> (88) Date of publication of the international search report: 1 August 1996 (01.08.96)
(54) Title: METHOD FOR PRODUCING BIOLOGICALS IN PROTEIN-FREE CULTURE		
(57) Abstract <p>The present invention includes an approach for producing viruses, such as Influenza, and vaccines derived therefrom as well as recombinant proteins derived from viral vectors, by utilizing vertebrate cells cultured under protein-free conditions. These cells, which include a cellular biomass, show improved capabilities for propagating viruses, and eliminate the need for costly and time-consuming viral passaging and purification. The invention also includes further approaches for enhancing the propagation of virus by employing activating substances, modifying the activation site of viruses and using augmentation loops. Improved approaches for producing viral reassortants also are provided.</p>		

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INTERNATIONAL SEARCH REPORT

International Application No
PCT/EP 95/04439

A. CLASSIFICATION OF SUBJECT MATTER		
IPC 6	C12N7/00 C07K14/11	C12N5/00 A61K39/15
	A61K39/145 A61K39/245	C12P21/00 A61K39/12
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)		
IPC 6 C12N A61K C12P C07K		
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)		
C. DOCUMENTS CONSIDERED TO BE RELEVANT		
Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
A	ARCH. VIROL., vol.125, 1992 pages 327 - 330, XP002000992 J. CINATL ET AL. 'Increased HIV-1 production in chronically infected H9 cells grown in protein-free medium' see the whole document ---	1-4
Y	EP,A,0 019 218 (CUTTER LABORATORIES INC.) 26 November 1980 see the whole document --- -/--	1-37
☒ Further documents are listed in the continuation of box C. ☒ Patent family members are listed in 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		Date of mailing of the international search report
19 April 1996		17. 06. 96
Name and mailing address of the ISA European Patent Office, P.B. 5818 Patentlaan 2 NL - 2280 HV Rijswijk Tel. (+31-70) 340-2040, Tx. 31 651 epo nl, Fax (+31-70) 340-3016		Authorized officer SKELLY J.M.

INTERNATIONAL SEARCH REPORT

International Application No
PCT/EP 95/04439

C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT

Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
Y	J. VIROL., vol.188, no.1, 1992 pages 408 - 411, XP002001034 M. VEY ET AL. 'Haemagglutinin activation of pathogenic avian influenza viruses of serotype H7 requires the protease recognition motif RXK/RR' cited in the application see the whole document ---	6
Y	BIOLOGICAL ABSTRACTS, vol. 96 1993, Philadelphia, PA, US; abstract no. 143083, J. CINATL ET AL. 'Protein-free culture of vero cells a substrate for replication of human pathogenic viruses' see abstract & CELL. BIOL. INT., vol.17, no.9, 1993 pages 885 - 895 ---	1-37, 51-65
Y	EP,A,0 113 665 (THE WELLCOME FOUNDATION LTD) 18 July 1984 see page 4 - page 5 ---	51-65
A	EP,A,0 485 689 (RIKAGAKU KENKYUSHO) 20 May 1992 ---	
P,A	INTERVIROLOGY, vol.37, 1994 pages 361 - 366, XP002000993 J. CINATL ET AL. 'Suspension culture of hela cells in protein-free medium: sensitivity to human pathogenic viruses' see the whole document -----	1-4

INTERNATIONAL SEARCH REPORT

International application No.

PCT/EP 95/04439

Box I Observations where certain claims were found unsearchable (Continuation of item 1 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 II Observations where unity of invention is lacking (Continuation of item 2 of first sheet)

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

14 inventions - see continuation-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 fee, this Authority did not invite payment of any additional fee.

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.:

see continuation-sheet, first invention

Remark on Protest

- ☐ The additional search fees were accompanied by the applicant's protest.
- ☐ No protest accompanied the payment of additional search fees.

FURTHER INFORMATION CONTINUED FROM PCT/ISA/210

1. Claims 1-4 (partially), 5, 6, 7-11 (partially), 12-14, 15-26 (partially), 27, 28-36 (partially), 37, 51-65. The problem is the provision of virus, virus antigens and the corresponding vaccines, free of contaminating proteins. The provision of viral antigens specifically from the family Orthomyxoviridae, free from contaminating proteins, including the production of donor virus for making reassortants. The solution is the production of viral antigens, more specifically Orthomyxoviral antigens, in cells cultivated in protein-free cell culture medium.

2. Claims 1-4 (partially), 7-11 (partially), 15-37 (partially), 15-26 (partially), 28-36 (partially). As for 1, the virus being from the family Paramyxoviridae.

3. Claims 1-4 (partially), 7-11 (partially), 15-37 (partially), 15-26 (partially), 28-30 (partially), 32-36 (partially). As for 1, the virus being from the family Reoviridae.

4. Claims 1-4 (partially), 7-11 (partially), 15-37 (partially), 15-25 (partially), 28-36, (partially), 40. As for 1, the virus being from the family Picornaviridae.

5. Claims 1-4 (partially), 7-11 (partially), 15-37 (partially), 15-25 (partially), 28-36 (partially), 38. As for 1, the virus being from the family Flaviviridae.

6. Claims 1-4 (partially), 7-11 (partially), 15-37 (partially), 15-25 (partially), 28-30 (partially), 32-36 (partially). As for 1, the virus being from the family Arenaviridae.

7. Claims 1-4 (partially), 7-11 (partially), 15-37 (partially), 15-25 (partially), 28-36 (partially), 39. As for 1, the virus being from the family Herpesviridae.

FURTHER INFORMATION CONTINUED FROM PCT/ISA/210

8. Claims 1-4 (partially), 7-11 (partially), 15-37 (partially), 15-25 (partially), 28-30 (partially), 32-36 (partially). As for 1, the virus being from the family Poxviridae.

9. Claims 1-4 (partially), 7-11 (partially), 15-37 (partially), 15-25 (partially), 28-30 (partially), 32-36 (partially). As for 1, the virus being from the family Adenoviridae.

10. Claims 41-44: The problem is the provision of further recombinant proteins free of contaminating proteins. The solution is their production in cells cultivated in protein-free medium.

11. Claims 45-50: The problem is the production of a further cellular biomass. The solution is growth of the cells on a carrier in a protein-free medium.

12. Claim 66-68: The problem is to provide means for selecting reassortant viruses. The solution is antibodies which bind to a donor virus.

13. Claims 69, 70: The problem is to provide a method of increasing the infectivity of a virus that express a protein involved in virus activation. The solution is to use a virus with a modified cleavage site in its haemagglutinin.

14. Claim 71: The problem is to optimise the production of one or more products from cultured cells. The solution is the use of activating and attenuating compounds.

INTERNATIONAL SEARCH REPORT

Information on patent family members

International Application No

PCT/EP 95/04439

Patent document cited in search report	Publication date	Patent family member(s)	Publication date
EP-A-0019218	26-11-80	AR-A- 220838	28-11-80
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