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<p>(21) International Application Number: PCT/US97/08950</p> <p>(22) International Filing Date: 23 May 1997 (23.05.97)</p> <p>(30) Priority Data: 08/653,226 24 May 1996 (24.05.96) US Not furnished 20 May 1997 (20.05.97) US</p> <p>(71) Applicant: CHIRON CORPORATION [US/US]; 4560 Horton Street, Emeryville, CA 94608 (US).</p> <p>(72) Inventors: VALENZUELA, Pablo, D., T.; 2919 Avalon Avenue, Berkeley, CA 94705 (US). CHIEN, David, Ying; 1121 Douglas Court, Alamo, CA 94507 (US).</p> <p>(74) Agents: GOLDMAN, Kenneth, M.; Chiron Corporation, Intellectual Property - R440, P.O. Box 8097, Emeryville, CA 94662-8097 (US) et al.</p>		<p>(81) Designated States: AL, AM, AT, AU, AZ, BA, BB, BG, BR, BY, CA, CH, CN, CU, CZ, DE, DK, EE, ES, FI, GB, GE, HU, IL, IS, JP, KE, KG, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MD, MG, MK, MN, MW, MX, NO, NZ, PL, PT, RO, RU, SD, SE, SG, SI, SK, TJ, TM, TR, TT, UA, UG, UZ, VN, ARIPO patent (GH, KE, LS, MW, SD, SZ, UG), Eurasian patent (AM, AZ, BY, KG, KZ, MD, RU, TJ, TM), European patent (AT, BE, CH, DE, DK, ES, FI, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE), OAPI patent (BF, BJ, CF, CG, CI, CM, GA, GN, ML, MR, NE, SN, TD, TG).</p> <p>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></p> <p>(88) Date of publication of the international search report: 31 December 1997 (31.12.97)</p>

(54) Title: MULTIPLE EPITOPE FUSION PROTEIN

		MEFA-3 ANTIGEN									
hSOD- (1-154)		CORE	CORE	c33c	5-1-1 type 1	5-1-1 type 3	5-1-1 type 2	C-100	C-100	NSS	NSS
AMINO ACIDS	10	10	1192	1694	1694	1694	1901	1901	2278	2278	
		53	53	1457	1735	1735	1735	1940	1940	2310	2310

		MEFA-5 ANTIGEN										
hSOD- (1-154)		CORE	CORE	E1	E2	c33c	5-1-1 type 1	5-1-1 type 3	5-1-1 type 2	C-100	NSS	NSS
AMINO ACIDS	10	10	303	405	1192	1689	1689	1689	1689	1901	2278	2278
		53	53	320	444	1457	1735	1735	1735	1940	2313	2313

		MEFA-6 ANTIGEN										
hSOD- (1-154)		E1	E2	c33c	5-1-1 type 1	5-1-1 type 3	5-1-1 type 2	C-100	NSS	NSS	CORE	CORE
AMINO ACIDS	303	405	1192	1689	1689	1689	1689	1901	2278	2278	10	10
		320	444	1457	1735	1735	1735	1940	2313	2313	53	53

(57) Abstract

Multiple copy epitope immunoassays are produced by: (1) identifying nucleotide sequences that encode a plurality of different epitopes; (2) placing the nucleotide sequences into an expression cassette wherein at least two copies of a sequence coding for the same epitope, preferably from different strains of a pathogen, are placed in the cassette; (3) transforming a suitable host with the cassette in order to express the sequences encoding the epitopes; (4) purifying the expressed epitopes; and (5) coating the epitopes on a surface of a substrate. The purified epitopes are encompassed by the general structural formula (A)_x-(B)_y-(C)_z which represents a linear amino acid sequence, B is an amino acid sequence of an epitope or cluster of epitopes and each B contains at least five and not more than 1,000 amino acids, y is an integer of 2 or more, A and C are each independently an amino acid sequence of an epitope or cluster of epitopes not adjacent to B in nature and x and z are each independently an integer of 0 or more wherein at least one of x and z is 1 or more. The epitopes of the invention are more soluble than and are therefore more easily purified than conventional epitopes. Further, the presence of repeating epitope sequences (repeating at least B in the same linear amino acid sequence from different strains of a pathogen) increases the sensitivity and specificity of the assay. Repeated epitope sequences in a single linear antigen also decreases masking problems and makes it possible to include a greater number of epitopes on a unit area of substrate thereby improving sensitivity in the detection of antibodies.

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INTERNATIONAL SEARCH REPORT

International Application No
PCT/US 97/08950

A. CLASSIFICATION OF SUBJECT MATTER
 IPC 6 C12N15/62 C12N15/51 C12N15/48 C07K14/18 G01N33/50
 C12Q1/68

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 C07K G01N C12Q

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.
X	SMYTHE JA ET AL: "Production of linear polymers of HIV gp120-binding domains [letter]" PROTEIN ENG, FEB 1994, 7 (2) P145-7, ENGLAND, XP002044415 see the whole document --- -/--	6,7

Further documents are listed in the continuation of box C.

Patent family members are listed in annex.

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Date of the actual completion of the international search 23 October 1997	Date of mailing of the international search report 10. 11. 97
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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 Espen, J
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INTERNATIONAL SEARCH REPORT

Intern. Application No

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C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT

Category °	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	<p>CHIEN D.Y. ET AL: "Diagnosis of hepatitis C virus (HCV) infection using an immunodominant chimeric polyprotein to capture circulating antibodies: Reevaluation of the role of HCV in liver disease"</p> <p>PROCEEDINGS OF THE NATIONAL ACADEMY OF SCIENCES OF USA., vol. 89, 1992, WASHINGTON US, pages 10011-10015, XP002044416 cited in the application</p>	6,7
Y	<p>see figure 1</p> <p>see page 10011, right-hand column - page 10012, left-hand column; figure 1</p>	1-4, 8-10,12
Y	<p>--- CHATTERJEE S ET AL: "Fine specificity of immune responses to epitopic sequences in synthetic peptides containing B and T epitopes from the conserved Plasmodium falciparum blood-stage antigens." VACCINE, 1995, 13 (15) P1474-81, ENGLAND, XP004057461 see table 1</p>	1-4, 8-10,12
Y	<p>--- VAN DER PLOEG, J. R. ET AL: "Immunological properties of multiple repeats of a linear epitope of herpes simplex virus type 1 glycoprotein D" J. IMMUNOL. METHODS, 1989, 124, 211-17, XP002044417 see tables I,III</p>	1-4, 8-10,12
X	<p>--- WO 93 00365 A (CHIRON CORP) 7 January 1993 cited in the application</p>	6,7
Y	<p>see the whole document</p>	1-4, 8-10,12
X	<p>--- WO 94 18234 A (UNITED BIOMEDICAL INC) 18 August 1994 see claims 1-9; figure 1</p>	6,7
Y	<p>--- GB 2 294 047 A (MERCK & CO INC) 17 April 1996 see claim 1</p>	1-4, 8-10,12
X	<p>--- WO 93 08280 A (IMMULOGIC PHARMA CORP) 29 April 1993</p>	6,7
Y	<p>see figures 5,6,10-12</p> <p>--- -/--</p>	1-4, 8-10,12

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