

(19) World Intellectual Property
Organization
International Bureau



(43) International Publication Date
13 January 2005 (13.01.2005)

PCT

(10) International Publication Number
WO 2005/003777 A3

(51) International Patent Classification⁷: **G01N 33/58**,
C07C 69/017, C07F 9/12, C07H 15/20, 15/24, 15/26

(21) International Application Number:
PCT/US2004/020700

(22) International Filing Date: 24 June 2004 (24.06.2004)

(25) Filing Language: English

(26) Publication Language: English

(30) Priority Data:
60/482,596 24 June 2003 (24.06.2003) US

(71) Applicant (for all designated States except US): **VENTANA MEDICAL SYSTEMS, INC.** [US/US]; 1910 Innovation Park Drive, Tucson, AZ 85737 (US).

(72) Inventors: **BIENIARZ, Christopher**; 2462 E. Calle Sin controversia, Tucson, AZ 85718 (US). **KERNAG, Casey, A.**; 1765 S. Jones Blvd., #P-10, Tucson, AZ 85713 (US). **KOSMEDER, Jerome, W.**; 5530 N. Moccasin Trail, Tucson, AZ 85750 (US). **RODGERS, Paula, M.**; 4338 E. Whitman Street, Tucson, AZ 85711 (US). **WONG, Jennifer**; 1019 E. Drachman, Tucson, AZ 85719 (US).

(74) Agent: **JONES, Huw, R.**; Ventana Medical Systems, Inc., 1910 Innovation Park, Tucson, AZ 85737 (US).

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

(84) Designated States (unless otherwise indicated, for every kind of regional protection available): ARIPO (BW, GH, GM, KE, LS, MW, MZ, NA, SD, SL, SZ, TZ, UG, ZM, ZW), Eurasian (AM, AZ, BY, KG, KZ, MD, RU, TJ, TM), European (AT, BE, BG, CH, CY, CZ, DE, DK, EE, ES, FI, FR, GB, GR, HU, IE, IT, LU, MC, NL, PL, PT, RO, SE, SI, SK, TR), OAPI (BF, BJ, CF, CG, CI, CM, GA, GN, GQ, GW, ML, MR, NE, SN, TD, TG).

Published:
— with international search report

(88) Date of publication of the international search report:
9 June 2005

For two-letter codes and other abbreviations, refer to the "Guidance Notes on Codes and Abbreviations" appearing at the beginning of each regular issue of the PCT Gazette.

(54) Title: ENZYME-CATALYZED METAL DEPOSITION FOR THE ENHANCED IN SITU DETECTION OF IMMUNOHISTOCHEMICAL EPITOPES AND NUCLEIC ACID SEQUENCES

(57) Abstract: The invention is directed to novel compositions of matter and methods of detecting *in situ* an immunohistochemical epitope or nucleic acid sequence of interest in a biological sample comprising binding an enzyme-labeled conjugate molecule to the epitope or sequence of interest in the presence of a redox-inactive reductive species and a soluble metal ion, thereby facilitating the reduction of the metal ion to a metal atom at or about the point where the enzyme is anchored. Novel phosphate derivatives of reducing agents are described that when exposed to a phosphatase are activated to their reducing form, thereby reducing metal ions to insoluble metal.



WO 2005/003777 A3

INTERNATIONAL SEARCH REPORT

International Application No

PCT/US2004/020700

A. CLASSIFICATION OF SUBJECT MATTER

IPC 7 G01N33/58 C07C69/017 C07F9/12 C07H15/20 C07H15/24
C07H15/26

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 7 G01N C07C C07F C07H

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)

EPO-Internal, WPI Data, BIOSIS, EMBASE, PAJ

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category °	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
A	US 2002/142411 A1 (HAINFELD JAMES F) 3 October 2002 (2002-10-03) cited in the application the whole document -----	1-11, 18-32,36
A	US 5 116 734 A (HIGGS THOMAS W ET AL) 26 May 1992 (1992-05-26) cited in the application the whole document -----	1-11, 18-32,36
A	WO 03/035900 A (BURTON MICHAEL) 1 May 2003 (2003-05-01) the whole document -----	1-11, 18-32,36
A	US 5 073 483 A (LEBACQ PHILIPPE) 17 December 1991 (1991-12-17) the whole document ----- -/--	1-11, 18-32,36

☒ 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

19 November 2004

Date of mailing of the international search report

22. 03. 05

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

Döpfer, K-P

INTERNATIONAL SEARCH REPORT

International Application No

PCT/JP2004/020700

C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT

Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
A	PATTON W F: "Detection technologies in proteome analysis" JOURNAL OF CHROMATOGRAPHY B: BIOMEDICAL SCIENCES & APPLICATIONS, ELSEVIER SCIENCE PUBLISHERS, NL, vol. 771, no. 1-2, 5 May 2002 (2002-05-05), pages 3-31, XP004350527 ISSN: 1570-0232 page 4, right-hand column, line 1 - page 5, left-hand column, last line	1-11, 18-32,36
A	----- DATABASE BIOSIS [Online] BIOSCIENCES INFORMATION SERVICE, PHILADELPHIA, PA, US; 1983, MEUR S K ET AL: "A NEW TECHNIQUE FOR LOCALIZATION OF CELLULASE IN-SITU USING SILVER NITRATE" XP002305553 Database accession no. PREV198477046809 abstract & STAIN TECHNOLOGY, vol. 58, no. 2, 1983, pages 97-100, ISSN: 0038-9153	1-11
A	----- DATABASE BIOSIS [Online] BIOSCIENCES INFORMATION SERVICE, PHILADELPHIA, PA, US; 1984, PARTANEN S: "A DIRECT COLORING METAL PRECIPITATION METHOD FOR THE DEMONSTRATION OF ARYL SULFATASE A AND ARYL SULFATASE B" XP002305554 Database accession no. PREV198478052564 abstract & HISTOCHEMICAL JOURNAL, vol. 16, no. 5, 1984, pages 501-506, ISSN: 0018-2214 -----	1-11

INTERNATIONAL SEARCH REPORT

International application No.
PCT/US2004/020700

Box 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 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 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.:

1-11, 18-32, 36

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

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

1. claims: 1-11,18-32,36

Method for detecting in situ an immunohistochemical epitope or for in situ staining a biological sample having an epitope or nucleotide sequence of interest; kit for performing said method

2. claims: 14,33

Sesamol phosphate esters and sesamol glycosides; 0-(beta-lactamyl)-sesamol compounds

3. claims: 16,17

chromanol glycosides ; chromanol phosphate esters; 0-(beta-lactamyl)-chromanol compounds

4. claims: 15,34,35

Eugenol phosphate esters; eugenol glucosides; eugenol galactosides; 0-(beta-lactamyl)-eugenol compounds

5. claims: 12,13

anthrahydroquinone phosphate esters, anthrahydroquinone glucosides and galactosides;0-(beta-lactamyl)- anthrahydroquinone compounds

INTERNATIONAL SEARCH REPORT

Information on patent family members

International Application No

PCT/US2004/020700

Patent document cited in search report		Publication date	Patent family member(s)	Publication date
US 2002142411	A1	03-10-2002	NONE	
US 5116734	A	26-05-1992	AU 6295890 A WO 9103718 A1	08-04-1991 21-03-1991
WO 03035900	A	01-05-2003	EP 1438423 A1 WO 03035900 A1 US 2004235081 A1	21-07-2004 01-05-2003 25-11-2004
US 5073483	A	17-12-1991	FR 2629100 A1 FR 2680374 A1 AT 77654 T DE 68901878 D1 DE 68901878 T2 EP 0334756 A1 JP 2009400 A	29-09-1989 19-02-1993 15-07-1992 30-07-1992 17-12-1992 27-09-1989 12-01-1990