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(88) Date of publication of the international search report:
19 August 2004

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: ABSOLUTE QUANTIFICATION OF PROTEINS AND MODIFIED FORMS THEREOF BY MULTISTAGE MASS SPECTROMETRY

(57) Abstract: The invention provides reagents, kits and methods for detecting and/or quantifying proteins in complex mixtures, such as a cell lysate. The methods can be used in high throughput assays to profile cellular proteomes. In one aspect, the invention provides a peptide internal standard labeled with a stable isotope and corresponding in amino acid sequence to the amino acid sequence of a subsequence of a target polypeptide. In another aspect, the peptide internal standard is labeled at a modified amino acid residue and is used to determine the presence of, and/or quantitate the amount of a particular modified form of a protein.



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INTERNATIONAL SEARCH REPORT

International application No.

PCT/US02/25778

A. CLASSIFICATION OF SUBJECT MATTER

IPC(7) : G01N 33/53, 33/58

US CL : 435/5, 7, 188, 287, 294, 805, 810; 436/513, 532, 810; 422/55, 56, 57, 58, 61; 424/1, 8, 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)

U.S. : 435/5, 7, 188, 287, 294, 805, 810; 436/513, 532, 810; 422/55, 56, 57, 58, 61; 424/1, 8, 12;

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 practicable, search terms used)
Please See Continuation Sheet

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	ARNOTT et al. Rapid identification of comigrating gel-isolation proteins by ion trap-mass spectrometry. Electrophoresis. 1998. Vol. 19, pages 968-980. See entire document.	1-5 and 23-24
Y		6-19, 21-22 and 25-46
X	Ji et al. Strategy for qualitative and quantitative analysis in proteomics based on signature peptides. Journal of Chromatography B. 2000. Vol. 745, pages 197-210. See entire document.	1-5 and 23-24
Y		6-19, 21-22 and 25-46
X	LIN et al. C-Terminal peptide sequencing via multistage mass spectrometry. Analytical Chemistry. 1998. Vol. 70, pages 5162-5165. See entire document.	1-5 and 23-24
Y		6-19, 21-22 and 25-46
Y	CONRADS et al. Utility of accurate mass tags for proteome-wide protein identification. Analytical Chemistry, 2000. Vol. 72, pages 3349-3354. See entire document.	1-19 and 21-46
Y	US 4,444,879 A (FOSTER et al.) 24 April 1984 (24.04.1984), especially column 15.	41-46
A	MURBY et al. Differential degradation of a recombinant albumin-binding receptor. European Journal of Biochemistry, 1991, Vol. 199, pages 41-46. See entire document.	1-19 and 21-46

☐ 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 application or patent 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

"Z" document member of the same patent family

Date of the actual completion of the international search

31 October 2002 (31.10.2002)

Date of mailing of the international search report

31 MAY 2003

Name and mailing address of the ISA/US

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INTERNATIONAL SEARCH REPORT

International application No.

PCT/US02/25778

Box I Observations where certain claims were found unsearchable (Continuation of Item 1 of first sheet)

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

1. ☐ Claim Nos.:
because they relate to subject matter not required to be searched by this Authority, namely:
2. ☐ Claim 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. ☒ Claim Nos.: 20
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:

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.:

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

INTERNATIONAL SEARCH REPORT

PCT/US02/25778

Continuation of B. FIELDS SEARCHED Item 3:

STN- biosis, caplus, embase, medline, cancerlit, japio
EAST and WEST patent database