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(54) Title: DEVICE AND METHOD FOR HIGH-THROUGHPUT QUANTIFICATION OF MRNA FROM WHOLE BLOOD

(57) Abstract: Disclosed are a method, device kit, and automated system for simple, reproducible, and high-throughput quantification of mRNA from whole blood. More particularly, the method, device, kit and automated system involve combinations of leukocyte filters attached to oligo(dT)-immobilized multi-well plates.

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INTERNATIONAL SEARCH REPORT

International Application No
PCT/US2004/036309

A. CLASSIFICATION OF SUBJECT MATTER
IPC 7 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 7 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)

EPO-Internal, BIOSIS, MEDLINE, EMBASE, PAJ, 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 2003/157550 A1 (MASATO MITSUHASHI I.) 21 August 2003 (2003-08-21) claim 1 paragraphs [0024], [0028], [0031], [0041], [0058] -----	1,4-30
X	MIURA Y. ET AL: "Fluorometric determination of total mRNA with oligo (dT) immobilized on microtiter plates" CLINICAL CHEMISTRY, vol. 42, no. 11, 1996, pages 1758-1764, XP002326195 ISSN: 0009-9147	1
Y	page 1759, column 1, paragraph 4 - column 2, paragraph 2 page 1760, column 2, paragraph 2 figure 3 -----	4-24,29, 30
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[X] Further documents are listed in the continuation of box C.

[X] 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
9 May 2005	25. 07. 2005

Name and mailing address of the ISA	Authorized officer
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	Helliot, B

Form PCT/ISA/210 (second sheet) (January 2004)

INTERNATIONAL SEARCH REPORT

Internat[redacted]l 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	TAKASHI ISHIKAWA ET AL: "CONSTRUCTION OF CDNA BANK FROM BIOPSY SPECIMENS FOR MULTIPLE GENE ANALYSIS OF CANCER" CLINICAL CHEMISTRY, AMERICAN ASSOCIATION FOR CLINICAL CHEMISTRY. WINSTON, US, vol. 43, no. 5, 1997, pages 764-770, XP002917361 ISSN: 0009-9147	1
Y	page 765, column 2, paragraph 3 page 765, column 2, paragraph 5 - page 766, column 1, paragraph 1	4-24,29, 30
Y	ASO YOSHIMASA ET AL: "Rapid, stable ambient storage of leukocyte RNA from whole blood" CLINICAL CHEMISTRY, vol. 44, no. 8 PART 1, August 1998 (1998-08), pages 1782-1783, XP002326196 ISSN: 0009-9147 abstract	4-24,29, 30
A	WO 03/062462 A (DYNAL BIOTECH ASA; BOSNES, MARIE; DZIEGLEWSKA, HANNA) 31 July 2003 (2003-07-31) column 8, lines 50-55	
A	US 6 300 058 B1 (AKITAYA TATSUO ET AL) 9 October 2001 (2001-10-09) page 29, lines 26-29	

INTERNATIONAL SEARCH REPORT

International application No.
PCT/US2004/036309

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 (completely), 4-30 (completely)

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 (completely), 4-30 (completely)

A method of high throughput quantification of a specific mRNA in whole blood, comprising the steps of:
(a) collecting whole blood;
(b) removing erythrocytes and blood components other than leukocytes from the whole blood by filtration to yield leukocytes on a filter membrane;
(c) lysing the leukocytes on a filter membrane to produce a lysate comprising mRNA;
(d) transferring the lysate to an oligo(dT)-immobilized plate to capture the mRNA; and
(e) quantifying the specific mRNA using spiked control RNA.
A lysis buffer for high throughput mRNA quantification, comprising:
(a) a sufficient concentration of detergent to lyse a cytoplasmic membrane;
(b) a sufficient concentration of salt that the stringency does not exceed that of 4*SSC;
(c) a buffer to maintain pH in the range of 7.0-8.0;
(d) 1.4-1.75 M guanine thiocyanate; and
(e) 200 [mu]g/ml-20 mg/ml proteinase K.
A high throughput mRNA quantification kit, comprising:
(1) a high throughput mRNA quantification device comprising
(a) a multi-well plate, said multi-well plate comprising:
i) a plurality of sample-delivery wells;
ii) a leukocyte-capturing filter underneath said wells;
iii) an mRNA capture zone underneath said filter, said mRNA capture zone having oligo(dT)-immobilized thereon; and
(b) a vacuum box adapted to receive said plate to create a seal between said plate and said box.;
(2) a hypotonic buffer;
(3) ethanol; and
(4) a lysis buffer comprising 1.4-1.75 M guanine thiocyanate; and 200 [mu]g/ml -20 mg/ml proteinase K.
A method of lysing cells using the lysis buffer as disclosed herein above.

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2. claim: 2 (completely)

FURTHER INFORMATION CONTINUED FROM PCT/ISA/ 210

A method of high throughput quantification of a specific mRNA in whole blood, comprising the steps of:
(a) collecting whole blood;
(b) removing erythrocytes and blood components other than leukocytes from the whole blood by filtration to yield leukocytes on a filter membrane;
(c) lysing the leukocytes on a filter membrane to produce a lysate comprising mRNA;
(d) transferring the lysate to an oligo(dT)-immobilized plate to capture the mRNA while applying specific antisense primers; and
(e) quantifying the specific mRNA.

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3. claim: 3 (completely).

A method of high throughput quantification of a specific mRNA in whole blood, comprising the steps of:
(a) collecting whole blood;
(b) removing erythrocytes and blood components other than leukocytes from the whole blood by filtration to yield leukocytes on a filter membrane;
(c) lysing the leukocytes on a filter membrane to produce a lysate comprising mRNA;
(d) transferring the lysate to an oligo(dT)-immobilized plate to capture the mRNA; and
(e) quantifying the specific mRNA while applying specific antisense primers.

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4. claims: 31-51 (completely)

A method of determining a definite quantity of target mRNA in a blood sample comprising:
(a) collecting whole blood;
(b) removing erythrocytes and blood components other than leukocytes from the whole blood to yield leukocytes;
(c) lysing the leukocytes with a lysis buffer containing spiked control RNA to produce a lysate comprising mRNA and spiked control RNA;
(d) transferring the lysate to an oligo(dT)-immobilized plate to capture the mRNA;
(e) quantifying the sample mRNA and spiked control RNA;
(f) determining the percent recovery of spiked control RNA; and
(g) determining the definite quantity of mRNA by applying the percent recovery determined in step (f).

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5. claims: 52-53 (completely)

A method of synthesizing cDNA in solution upon poly-A RNA, comprising application of specific antisense primers during cDNA synthesis.

FURTHER INFORMATION CONTINUED FROM PCT/ISA/ 210

6. claims: 54-93 (completely)

A method for quantifying a first specific mRNA comprising a particular sequence from a sample, comprising:
a) spiking said sample with a known quantity of a second specific mRNA;
b) purifying poly-A mRNA from the sample;
c) producing cDNA from the mRNA in the sample;
d) quantifying an amount of cDNA corresponding to each of the first and second specific mRNA's in the sample;
e) determining a percent recovery of the second specific mRNA; and
f) applying the percent recovery of the second specific mRNA to determine the starting amount of the first specific mRNA.

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7. claims: 94-119 (completely)

A method of detecting mRNA indicative of a cellular response to a bioagent, comprising:
a) exposing cells to a bioactive agent;
b) lysing the cells with a lysis buffer comprising a known quantity of a specific control mRNA;
d) purifying poly-A mRNA from the lysate;
e) producing cDNA from a specific sequence of native mRNA and from the specific control mRNA;
f) quantifying an amount of cDNA corresponding to the specific native and specific control mRNAs;
g) creating a graph comprising the amount of recovered specific native mRNA on the y axis and the recovered specific control mRNA on the x axis; and
h) using the graph of step (g) to determine the amount of mRNA produced in response to exposure to a bioactive agent.

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8. claims: 120-145 (completely)

A method of detecting mRNA indicative of a cellular response to a bioagent, comprising:
a) exposing cells to a bioactive agent;
b) lysing the cells with a lysis buffer comprising a known quantity of a specific control mRNA;
c) purifying poly-A mRNA from the lysate;
d) producing cDNA from a specific sequence of native mRNA and from the specific control mRNA;
e) quantifying an amount of cDNA corresponding to the specific native and specific control mRNAs;
f) creating a graph comprising the amount of recovered specific native mRNA on the y axis and the recovered specific control mRNA on the x axis; and
g) comparing statistical differences among multiple points on the graph of step (f) to detect mRNA produced in response to exposure to a bioactive agent.

FURTHER INFORMATION CONTINUED FROM PCT/ISA/ 210

9. claims: 146-171 (completely)

A method of identifying an individual expressing abnormal levels of mRNA:
a) exposing cells to a bioactive agent;
b) lysing the cells with a lysis buffer comprising a known quantity of a specific control mRNA;
d) purifying poly-A mRNA from the lysate;
e) producing cDNA from a specific sequence of native mRNA and from the specific control mRNA;
f) quantifying an amount of cDNA corresponding to the specific native and specific control mRNAs;
g) creating a graph comprising the amount of recovered specific native mRNA on the y axis, the recovered specific control mRNA on the x axis, and a regression line;
h) rotating the x-axis of the graph of step (g) to align with the regression line;
i) determining the normal range of mRNA quantities from the graph of step (h); and
j) detecting the individuals with mRNA quantities falling outside of the range of normal mRNA quantities.

INTERNATIONAL SEARCH REPORT

mation on patent family members

International Application No
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