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**10727985.3 / 2 438 446**

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(54) **METHODS, REAGENTS AND KITS FOR FLOW CYTOMETRIC IMMUNOPHENOTYPING**

(57) The invention relates to the field of flow cytometry and more particularly to a panel of antibody reagents conjugated to fluorescent compounds. Provided are reagent compositions, comprising at least eight distinct fluorochrome-conjugated antibodies comprising a set of

at least three identification antibodies for the identification of a leukocyte population of interest and at least four characterization antibodies for further characterization and/or classification of said leukocyte population. Also provided are kits and methods related to the reagent compositions.

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EUROPEAN SEARCH REPORT

Application Number  
EP 17 16 5543

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DOCUMENTS CONSIDERED TO BE RELEVANT			
Category	Citation of document with indication, where appropriate, of relevant passages	Relevant to claim	CLASSIFICATION OF THE APPLICATION (IPC)
A	<p>BERHANU D ET AL: "Optimized lymphocyte isolation methods for analysis of chemokine receptor expression", JOURNAL OF IMMUNOLOGICAL METHODS, vol. 279, no. 1-2, 1 August 2003 (2003-08-01), pages 199-207, XP004455320, ELSEVIER SCIENCE PUBLISHERS B.V.,AMSTERDAM, NL ISSN: 0022-1759 * the whole document * * abstract * * page 202, right-hand column, last paragraph - page 203, left-hand column, paragraph 1 *</p> <p style="text-align: center;">-----</p>	1,3-12	<p>INV. G01N33/574 G01N33/58 G01N33/50 G01N15/14 G01N33/532 G01N33/533</p>
A	<p>BOULASSEL ET AL: "Immunophenotypic patterns of CD8&lt;+&gt; T cell subsets expressing CD8alphaalpha and IL-7Ralpha in viremic, aviremic and slow progressor HIV-1-infected subjects", CLINICAL IMMUNOLOGY, vol. 124, no. 2, 18 July 2007 (2007-07-18), pages 149-157, XP022156789, ACADEMIC PRESS, US ISSN: 1521-6616 * the whole document * * abstract * * page 150, right-hand column, last paragraph - page 151, left-hand column, paragraph 2 *</p> <p style="text-align: center;">-----</p> <p style="text-align: right;">-/--</p>	1,3-12	<p>TECHNICAL FIELDS SEARCHED (IPC)</p> <p>G01N</p>
<del>The present search report has been drawn up for all claims</del>			
Place of search		Date of completion of the search	Examiner
The Hague		13 June 2017	Gall-Truchot, A
CATEGORY OF CITED DOCUMENTS		<p>T : theory or principle underlying the invention E : earlier patent document, but published on, or after the filing date D : document cited in the application L : document cited for other reasons</p> <p>..... &amp; : member of the same patent family, corresponding document</p>	
<p>X : particularly relevant if taken alone Y : particularly relevant if combined with another document of the same category A : technological background O : non-written disclosure P : intermediate document</p>			

EPO FORM 1503 03.02 (P04C01)



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A	<p>ABRAMS BARNY ET AL:                      "3-Carboxy-6-chloro-7-hydroxycoumarin: a highly fluorescent, water-soluble violet-excitable dye for cell analysis.",                      ANALYTICAL BIOCHEMISTRY 15 MAR 2009, vol. 386, no. 2,                      15 March 2009 (2009-03-15), pages 262-269, XP025942189,                      ISSN: 1096-0309                      * the whole document *                      * abstract *                      * page 268, left-hand column, last paragraph - right-hand column, paragraph 1 *                      * figure 13 *</p>	1,3-12	
A	<p>BRAYLAN R C ET AL: "OPTIMAL NUMBER OF REAGENTS REQUIRED TO EVALUATE HEMATOLYMPHOID NEOPLASIAS: RESULTS OF AN INTERNATIONAL CONSENSUS MEETING",                      CYTOMETRY, vol. 46, no. 1,                      15 February 2001 (2001-02-15), pages 23-27, XP009050615,                      ALAN LISS, NEW YORK, US                      ISSN: 0196-4763                      * the whole document *                      * abstract *                      * page 26, left-hand column, paragraph 2; figure 1 *                      * page 27, left-hand column, paragraph 4 *</p>	1,3-12	
----- -/--			
<del>The present search report has been drawn up for all claims</del>			
Place of search <b>The Hague</b>		Date of completion of the search <b>13 June 2017</b>	Examiner <b>Gall-Truchot, A</b>
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A	CAMPANA D ET AL: "Immunophenotyping of leukemia", JOURNAL OF IMMUNOLOGICAL METHODS, vol. 243, no. 1-2, 21 September 2000 (2000-09-21), pages 59-75, XP004210693, ELSEVIER SCIENCE PUBLISHERS B.V.,AMSTERDAM, NL ISSN: 0022-1759 * the whole document * * abstract * * page 60, left-hand column, last paragraph - right-hand column, paragraph 1 *	1,3-12	
A	HAFERLACH T ET AL: "Modern diagnostics in acute leukemias", CRITICAL REVIEWS IN ONCOLOGY/HEMATOLOGY, ELSEVIER, AMSTERDAM, NL, vol. 56, no. 2, 1 November 2005 (2005-11-01), pages 223-234, XP027789944, ISSN: 1040-8428 [retrieved on 2005-11-01] * the whole document * * abstract * * table 6 *  ----- -/--	1,3-12	
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<del>The present search report has been drawn up for all claims</del>			
Place of search <b>The Hague</b>		Date of completion of the search <b>13 June 2017</b>	Examiner <b>Gall-Truchot, A</b>
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Category	Citation of document with indication, where appropriate, of relevant passages	Relevant to claim	CLASSIFICATION OF THE APPLICATION (IPC)
A	<p>DRACH D ET AL: "Flow cytometric detection of cytoplasmic antigens in acute leukemias: Implications for lineage assignment", LEUKEMIA RESEARCH, NEW YORK, NY, US, vol. 17, no. 5, 1 May 1993 (1993-05-01), pages 455-461, XP026290882, ISSN: 0145-2126, DOI: 10.1016/0145-2126(93)90102-Q [retrieved on 1993-05-01] * the whole document * * abstract *</p>	1,3-12	
A	<p>ANONYMOUS: "CONSENSUAL EUROPEAN IMMUNOPHENOTYPING PANELS FOR LEUKEMIA", 6TH PCRDT 23 April 2005 (2005-04-23), XP002556187, Retrieved from the Internet: URL:https://www.leukemia-net.org/content/diagnostics/diagnostics/documents/e5821/infoContent5822/WP10consensual_panels.pdf [retrieved on 2009-11-18] * the whole document * * page 2, paragraph 4 * * page 4 - page 5 *</p>	1,3-12	
----- -/--			
<del>The present search report has been drawn up for all claims</del>			
Place of search <b>The Hague</b>		Date of completion of the search <b>13 June 2017</b>	Examiner <b>Gall-Truchot, A</b>
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DOCUMENTS CONSIDERED TO BE RELEVANT			
Category	Citation of document with indication, where appropriate, of relevant passages	Relevant to claim	CLASSIFICATION OF THE APPLICATION (IPC)
T	<p>VAN DONGEN, JJM ET AL.: "EuroFlow antibody panels for standardized n-dimensional flow cytometric immunophotyping of normal, reactive and malignant leukocytes",                      4 June 2009 (2009-06-04), XP002555901,                      Retrieved from the Internet:                      URL:http://www.euroflow.org/imagenes/news/euroflow_handout_on_antibody_panels.pdf                      [retrieved on 2009-11-17]                      * the whole document *</p> <p>-----</p>		
T	<p>L LHERMITTE ET AL.: "A single 8-color flow-cytometry immunostaining allows delineation of both typical myeloid and lymphoid acute leukemia and undifferentiated/immature acute leukemia",                      4 June 2009 (2009-06-04), XP002555842,                      Retrieved from the Internet:                      URL:http://www.euroflow.org/imagenes/publications/lhermitte,_alot_poster,_eha_2009,_berlin.pdf                      [retrieved on 2009-11-17]                      * the whole document *</p> <p>-----</p>		<p>TECHNICAL FIELDS SEARCHED (IPC)</p>
<del>The present search report has been drawn up for all claims</del>			
Place of search <b>The Hague</b>		Date of completion of the search <b>13 June 2017</b>	Examiner <b>Gall-Truchot, A</b>
<p>CATEGORY OF CITED DOCUMENTS</p> <p>X : particularly relevant if taken alone                      Y : particularly relevant if combined with another document of the same category                      A : technological background                      O : non-written disclosure                      P : intermediate document</p>		<p>T : theory or principle underlying the invention                      E : earlier patent document, but published on, or after the filing date                      D : document cited in the application                      L : document cited for other reasons                      .....                      &amp; : member of the same patent family, corresponding document</p>	

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**CLAIMS INCURRING FEES**

The present European patent application comprised at the time of filing claims for which payment was due.

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Only part of the claims have been paid within the prescribed time limit. The present European search report has been drawn up for those claims for which no payment was due and for those claims for which claims fees have been paid, namely claim(s):

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No claims fees have been paid within the prescribed time limit. The present European search report has been drawn up for those claims for which no payment was due.

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**LACK OF UNITY OF INVENTION**

The Search Division considers that the present European patent application does not comply with the requirements of unity of invention and relates to several inventions or groups of inventions, namely:

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see sheet B

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All further search fees have been paid within the fixed time limit. The present European search report has been drawn up for all claims.

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As all searchable claims could be searched without effort justifying an additional fee, the Search Division did not invite payment of any additional fee.

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Only part of the further search fees have been paid within the fixed time limit. The present European search report has been drawn up for those parts of the European patent application which relate to the inventions in respect of which search fees have been paid, namely claims:

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None of the further search fees have been paid within the fixed time limit. The present European search report has been drawn up for those parts of the European patent application which relate to the invention first mentioned in the claims, namely claims:

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3(completely); 1, 4-12(partially)

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The present supplementary European search report has been drawn up for those parts of the European patent application which relate to the invention first mentioned in the claims (Rule 164 (1) EPC).



**LACK OF UNITY OF INVENTION  
SHEET B**

Application Number

EP 17 16 5543

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The Search Division considers that the present European patent application does not comply with the requirements of unity of invention and relates to several inventions or groups of inventions, namely:

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1. claims: 3(completely); 1, 4-12(partially)

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reagent compositions, diagnostic kits and methods for flow cytometric immuno-phenotyping of leukocytes comprising at least eight distinct fluorochrome-conjugated antibodies, wherein the antibodies are directed against the following combination of markers: cyCD3, CD45, cyMPO, cyCD79a, CD34, CD19, CD7 and CD3 (combination (a) of independent claim 1);

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2. claims: 2(completely); 1, 4-12(partially)

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reagent compositions, diagnostic kits and methods for flow cytometric immuno-phenotyping of leukocytes comprising at least eight distinct fluorochrome-conjugated antibodies, wherein the antibodies are directed against the following combination of markers: CD20, CD45, CD8, Ig[lambda], CD56, Ig[kappa], CD4, CD3, CD14 and CD38 (combination (b) of independent claim 1), wherein the antibody within the pairs CD8/Ig[lambda], CD56/Ig[kappa] and CD3/CD14 is conjugated to the same fluorochrome, and wherein between different pairs the fluorochromes are distinguishable (combination (b) of independent claim 1).

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专利名称(译)	用于流式细胞术免疫表型分析的方法，试剂和试剂盒		
公开(公告)号	<a href="#">EP3206031A3</a>	公开(公告)日	2017-10-25
申请号	EP2017165543	申请日	2010-06-02
[标]申请(专利权)人(译)	鹿特丹伊拉斯谟大学医疗中心		
申请(专利权)人(译)	伊拉斯姆斯大学医学中心鹿特丹		
当前申请(专利权)人(译)	伊拉斯姆斯大学医学中心鹿特丹		
[标]发明人	VAN DONGEN JACOBUS JOHANNES MARIA ORFAO DE MATOS CORREIA E VALE JOSE ALBERTO FLORES MONTERO JUAN ALEJANDRO ALMEIDA PARRA JULIA MARIA VAN DER VELDEN VINCENT HENRICUS JOHANNES BOTTCHER SEBASTIAN RAWSTRON ANDREW CRAIG DE TUTE RUTH MARY LHERMITTE LUDOVIC BERNARD SIMON ASNAFI VAHID MEJSTRIKOVA ESTER SZCZEPANSKI TOMASZ MONTEIRO DA SILVA LUCIO PAULO JORGE AYUSO MARTA MARIN PEDREIRA CARLOS EDUARDO		
发明人	VAN DONGEN, JACOBUS JOHANNES MARIA ORFAO DE MATOS CORREIA E VALE, JOSÉ ALBERTO FLORES MONTERO, JUAN ALEJANDRO ALMEIDA PARRA, JULIA MARIA VAN DER VELDEN, VINCENT HENRICUS JOHANNES BÖTTCHER, SEBASTIAN RAWSTRON, ANDREW CRAIG DE TUTE, RUTH MARY LHERMITTE, LUDOVIC BERNARD SIMON ASNAFI, VAHID MEJSTRÍKOVÁ, ESTER SZCZEPANSKI, TOMASZ MONTEIRO DA SILVA LUCIO, PAULO JORGE AYUSO, MARTA MARÍN PEDREIRA, CARLOS EDUARDO		
IPC分类号	G01N33/574 G01N33/58 G01N33/50 G01N15/14 G01N33/532 G01N33/533		
CPC分类号	G01N33/5094 G01N15/14 G01N33/5005 G01N33/5091 G01N33/532 G01N33/533 G01N2015/1402 G01N2015/1413		
优先权	2009161870 2009-06-03 EP PCT/NL2010/050332 2010-06-02 WO		
其他公开文献	EP3206031B1 EP3206031A2		
外部链接	<a href="#">Espacenet</a>		

摘要(译)

本发明涉及流式细胞术领域，更具体地涉及与荧光化合物缀合的一组抗体试剂。提供了试剂组合物，其包含至少八种不同的荧光染料缀合的抗体，其包含一组至少三种鉴定抗体，用于鉴定目的白细胞群和至少四种表征抗体，用于进一步表征和/或分类所述白细胞群。还提供了与试剂组合物相关的试剂盒和方法。



DOCUMENTS CONSIDERED TO BE RELEVANT		
Category	Citation of document with indication, where appropriate, of relevant passages	Relevant location
A	BERHANU D ET AL: "Optimized lymphocyte isolation methods for analysis of chemokine receptor expression", JOURNAL OF IMMUNOLOGICAL METHODS, vol. 279, no. 1-2, 1 August 2003 (2003-08-01), pages 199-207, XP004455320, ELSEVIER SCIENCE PUBLISHERS B.V., AMSTERDAM, NL ISSN: 0022-1759 * the whole document * * abstract * * page 202, right-hand column, last paragraph - page 203, left-hand column, paragraph 1 *	1,3-12 INV. G01N33/574 G01N33/58 G01N33/59 G01N15/14 G01N33/532 G01N33/533
A	BOULASSEL ET AL: "Immunophenotypic patterns of CD8 <sup>+</sup> T cell subsets expressing CD8alpha alpha and IL-7alpha in viremic, aviremic and slow progressor HIV-1 infected subjects", CLINICAL IMMUNOLOGY, vol. 124, no. 2, 18 July 2007 (2007-07-18), pages 149-157, XP022156789, ACADEMIC PRESS, US ISSN: 1521-6616 * the whole document * * abstract * * page 150, right-hand column, last paragraph - page 151, left-hand column, paragraph 2 *	1,3-12 TECHNICAL FIELD (IPC) G01N
-The present search report has been drawn up for all claims-		
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Date of search		Date of publication of the claims
13 June 2017		
CATEGORY OF CITED DOCUMENTS		
<input checked="" type="checkbox"/> particularly relevant to the claims <input checked="" type="checkbox"/> document of the same category with another <input checked="" type="checkbox"/> non-patent literature <input checked="" type="checkbox"/> non-scientific literature <input type="checkbox"/> intermediate document		
<input type="checkbox"/> library or archive underlying the invention <input type="checkbox"/> patent document, but not under the <input type="checkbox"/> application <input type="checkbox"/> document related to other reasons <input type="checkbox"/> number of the same patent family, corresponding document		