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

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[Continued on next page]

(54) Title: METHOD OF REVERSIBLY STAINING A TARGET CELL

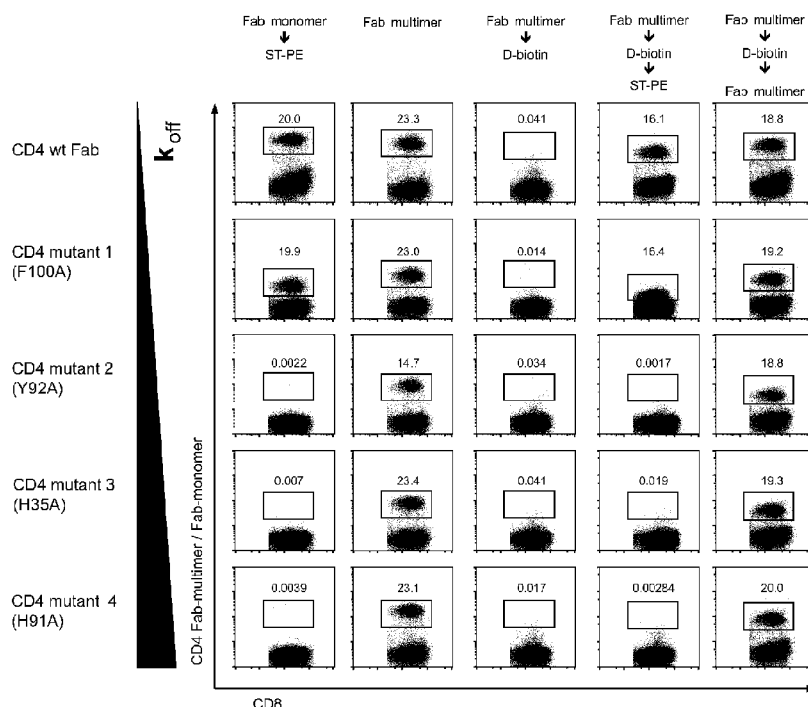


Fig. 2

(57) Abstract: The present invention relates to methods of reversibly staining a target cell. The invention also relates to methods of isolating a target cell or a target cell population that is defined by the presence of at least one common specific receptor molecule. The invention also provides kits that can be used to carry out the methods of the invention.



UG, ZM, ZW), Eurasian (AM, AZ, BY, KG, KZ, RU, TJ, TM), European (AL, AT, BE, BG, CH, CY, CZ, DE, DK, EE, ES, FI, FR, GB, GR, HR, HU, IE, IS, IT, LT, LU, LV, MC, MK, MT, NL, NO, PL, PT, RO, RS, SE, SI, SK, SM, TR), OAPI (BF, BJ, CF, CG, CI, CM, GA, GN, GQ, GW, ML, MR, NE, SN, TD, TG).

— *before the expiration of the time limit for amending the claims and to be republished in the event of receipt of amendments (Rule 48.2(h))*

(88) Date of publication of the international search report:

2 May 2013

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— *with international search report (Art. 21(3))*

INTERNATIONAL SEARCH REPORT

International application No.

PCT/EP2012/063969

Box No. I Nucleotide and/or amino acid sequence(s) (Continuation of item 1.c of the first sheet)

1. With regard to any nucleotide and/or amino acid sequence disclosed in the international application and necessary to the claimed invention, the international search was carried out on the basis of:
 - a. (means)

☐

 on paper
 - ☒

 in electronic form
 - b. (time)

☐

 in the international application as filed
 - ☐

 together with the international application in electronic form
 - ☒

 subsequently to this Authority for the purpose of search
2.

☒

 In addition, in the case that more than one version or copy of a sequence listing and/or table relating thereto has been filed or furnished, the required statements that the information in the subsequent or additional copies is identical to that in the application as filed or does not go beyond the application as filed, as appropriate, were furnished.
3. Additional comments:

INTERNATIONAL SEARCH REPORT

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Box No. 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 No. 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 fees, this Authority did not invite payment of additional fees.
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.:

1-36

Remark on Protest

- ☐ The additional search fees were accompanied by the applicant's protest and, where applicable, the payment of a protest fee.
- ☐ The additional search fees were accompanied by the applicant's protest but the applicable protest fee was not paid within the time limit specified in the invitation.
- ☐ No protest accompanied the payment of additional search fees.

INTERNATIONAL SEARCH REPORT

International application No
PCT/EP2012/063969

A. CLASSIFICATION OF SUBJECT MATTER
INV. C07K14/36 G01N33/50
ADD.

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)
C07K G01N

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)

EPO-Internal, BIOSIS, Sequence Search, EMBASE, 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	Tim Schroeder: "Nach Zellen angeln", Faszination Forschung, 30 June 2010 (2010-06-30), pages 28-37, XP002687294, Retrieved from the Internet: URL: http://www.google.de/url?sa=t&rct=j&q=%22fab-strep%22%20stemberger&source=web&cd=7&ved=0CD4QFjAG&url=http%3A%2F%2Fportal.mytum.de%2Fpressestelle%2Ffaszination-forschung%2F2010nr6%2F05_Zellen.pdf%2Fdownload&ei=APu1UPW3DMWw0QWa4oDgDA&usg=AFQjCNGwSHKQR7PkflZIEou_V9PZ6Nz-Ng&cad=rja [retrieved on 2012-11-16] the whole document ----- -/-	1-11, 15-18, 22-24, 26-36



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 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

16 November 2012

Date of mailing of the international search report

12/03/2013

Name and mailing address of the ISA/

European Patent Office, P.B. 5818 Patentlaan 2
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Authorized officer

Offermann, Stefanie

INTERNATIONAL SEARCH REPORT

International application No

PCT/EP2012/063969

C(Continuation). DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	ANITA SCHMITT ET AL: "Adoptive transfer and selective reconstitution of streptamer-selected cytomegalovirus-specific CD8+ T?cells leads to virus clearance in patients after allogeneic peripheral blood stem cell transplantation", TRANSFUSION, vol. 51, no. 3, 6 December 2010 (2010-12-06), pages 591-599, XP055043927, ISSN: 0041-1132, DOI: 10.1111/j.1537-2995.2010.02940.x	1-11,15, 22,26-32
Y	the whole document	12-14, 16-21, 23-25, 33-36
X	----- KNABEL MICHAEL ET AL: "Reversible MHC multimer staining for functional isolation of T-cell populations and effective adoptive transfer", NATURE MEDICINE, NATURE PUBLISHING GROUP, NEW YORK, NY, US, vol. 8, no. 6, 1 June 2002 (2002-06-01), pages 631-637, XP002460640, ISSN: 1078-8956, DOI: 10.1038/NM0602-631	1-11,15, 22,26-32
Y	the whole document figure 3b	12-14, 16-21, 23-25, 33-36
Y	----- MATSUI K ET AL: "KINETICS OF T-CELL RECEPTOR BINDING TO PEPTIDE/I-EK COMPLEXES CORRELATION OF THE DISSOCIATION RATE WITH T-CELL RESPONSIVENESS", PROCEEDINGS OF THE NATIONAL ACADEMY OF SCIENCES, NATIONAL ACADEMY OF SCIENCES, US, vol. 91, 1 December 1994 (1994-12-01), pages 12862-12866, XP002008363, ISSN: 0027-8424, DOI: 10.1073/PNAS.91.26.12862 abstract ----- -/--	1-36

INTERNATIONAL SEARCH REPORT

International application No

PCT/EP2012/063969

C(Continuation). DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	ODENDAHL M K ET AL: "GMP COMPLIANT ISOLATION OF UNTOUCHED CMV-SPECIFIC DONOR DERIVED CD3+CD8+T LYMPHOCYTES USING STREPTAMER-TECHNOLOGY", VOX SANGUINIS, S. KARGER AG, BASEL, CH, vol. 99, no. Suppl.1, 1 June 2010 (2010-06-01), pages 490-491, XP009164629, ISSN: 0042-9007 [retrieved on 2010-06-25]	1-11,15, 22,26-32
Y	the whole document	12-14, 16-21, 23-25, 33-36
X	----- ROSAELY CASALEGNO-GARDUÑA Â+-0 ET AL: "Multimer technologies for detection and adoptive transfer of antigen-specific T cells", CANCER IMMUNOLOGY, IMMUNOTHERAPY, SPRINGER, BERLIN, DE, vol. 59, no. 2, 22 October 2009 (2009-10-22), pages 195-202, XP019757721, ISSN: 1432-0851	1-11,15, 22,26-32
Y	the whole document page 197; figure 2a	12-14, 16-21, 23-25, 33-36
X	----- NEUDORFER JULIA ET AL: "Reversible HLA multimers (Streptamers) for the isolation of human cytotoxic T lymphocytes functionally active against tumor- and virus-derived antigens", JOURNAL OF IMMUNOLOGICAL METHODS, ELSEVIER SCIENCE PUBLISHERS B.V.,AMSTERDAM, NL, vol. 320, no. 1-2, 30 March 2007 (2007-03-30), pages 119-131, XP002518434, ISSN: 0022-1759, DOI: 10.1016/J.JIM.2007.01.001	1-11,15, 22,26-32
Y	the whole document	12-14, 16-21, 23-25, 33-36
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INTERNATIONAL SEARCH REPORT

International application No

PCT/EP2012/063969

C(Continuation). DOCUMENTS CONSIDERED TO BE RELEVANT		
Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
Y	SCHMIDT THOMAS G M ET AL: "The Strep-tag system for one-step purification and high-affinity detection or capturing of proteins", NATURE PROTOCOLS, NATURE PUBLISHING GROUP, GB, vol. 2, no. 6, 1 January 2007 (2007-01-01), pages 1528-1535, XP001536704, ISSN: 1750-2799, DOI: 10.1038/NPROT.2007.209 the whole document	1-36
X	US 5 985 658 A (COLINAS ROBERT JAMES [US] ET AL) 16 November 1999 (1999-11-16) the whole document	1-8,12, 26-32
X	RÃ Â CR GIS BOUQUIÃ Â CR ET AL: "A fast and efficient HLA multimer-based sorting procedure that induces little apoptosis to isolate clinical grade human tumor specific T lymphocytes", CANCER IMMUNOLOGY, IMMUNOTHERAPY, SPRINGER, BERLIN, DE, vol. 58, no. 4, 27 August 2008 (2008-08-27), pages 553-566, XP019706296, ISSN: 1432-0851 the whole document	1-8,17, 22,26-32
X	WO 02/054065 A2 (IBA GMBH [DE]; BUSCH DIRK H [DE]; WAGNER HERMANN [DE]) 11 July 2002 (2002-07-11) the whole document	1-36
T	CHRISTIAN STEMBERGER ET AL: "Novel Serial Positive Enrichment Technology Enables Clinical Multiparameter Cell Sorting", PLOS ONE, vol. 7, no. 4, 24 April 2012 (2012-04-24), page e35798, XP055043969, DOI: 10.1371/journal.pone.0035798	

INTERNATIONAL SEARCH REPORT

Information on patent family members

International application No

PCT/EP2012/063969

Patent document cited in search report	Publication date	Patent family member(s)	Publication date
US 5985658	A	16-11-1999	NONE

WO 02054065	A2	11-07-2002	CA 2467434 A1 11-07-2002
			EP 1227321 A1 31-07-2002
			EP 1346217 A2 24-09-2003
			JP 4416400 B2 17-02-2010
			JP 2004525354 A 19-08-2004
			US 2004082012 A1 29-04-2004
			US 2011070605 A1 24-03-2011
			WO 02054065 A2 11-07-2002

FURTHER INFORMATION CONTINUED FROM PCT/ISA/ 210

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

1. claims: 1-36

method of reversibly staining a target cell using a receptor binding reagent and a multimerization reagent as defined in claim 1

2. claims: 37-52

method of reversibly staining a target cell using a receptor binding reagent and a multimerization reagent as defined in claim 1

3. claims: 53, 54

kit for carrying out the method of invention I

4. claims: 55-59

use of a receptor binding reagent for a method of reversibly staining a target cell as defined in invention I

5. claims: 60, 61

method of reversibly staining a target cell using at least two receptor binding reagent and a multimerization reagent as defined in claim 60

6. claims: 62, 63

kit for carrying out the method of invention V
