ePCT - Cover Letter

ePCT Action: Submit Chapter II Demand

Competent IPEA:

SG

IA Number:

PCT/SG2017/050500

The following documents were submitted as attachments:

- Chapter II Demand : 1 document
- Letter accompanying amendments under Article 34: 1 document
- Article 34 Amendments: 4 documents

User Name:	MICHAEL GERARD MCLAUGHLIN
Date:	26 March 2018 11:24:51 CEST
Authentication:	Sign-in with strong authentication
Signature:	/MICHAEL GERARD MCLAUGHLIN//

PCT

DEMAND

under Article 31 of the Patent Cooperation Treaty:
The undersigned requests that the international application specified below be the subject of international preliminary examination according to the Patent Cooperation Treaty.

For International Preliminary Examining Authority use only

Identification of IPEA: SG	Date of receipt of DEMAND: 26 March 2018 (26.03.2018)

Box No. I IDENTIFICATION OF THE INTERNATIONAL APPLICATION		Applicant's or agent's file reference P1007-WO
International application No. PCT/SG2017/050500	International filing date 05 October 2017 (05.10.2017)	(Earliest) Priority date 05 October 2016 (05.10.2016)
Title of invention A METHOD OF ISOLATING MESENCHYMAL STEM CELLS FROM UMBILICAL CORD AMNIOTIC MEMBRANE USING A CELL CULTURE MEDIUM		

Box No. II APPLICANT(S)

Name and address: **CELLRESEARCH CORPORATION PTE. LTD.** **137 Market Street, #08-02** **Grace Global Raffles Building** **Singapore 048943** **Singapore**	Telephone No. / Facsimile No. / Applicant's registration No. with the Office
Nationality: Singapore	Residence: Singapore

E-mail authorization: Marking one of the check-boxes below authorizes the International Bureau and the International Preliminary Examining Authority to use the e-mail address indicated in this Box to send notifications issued in respect of this international application if those offices are willing to do so.

☐ as advance copies followed by paper notifications; or ☐ exclusively in electronic form (no paper notifications will be sent).

E-mail address:

☐ Further applicants are indicated on a continuation sheet.

Box No. III AGENT OR COMMON REPRESENTATIVE; OR ADDRESS FOR CORRESPONDENCE

The following person is ☑ agent ☐ common representative

and ☑ has been appointed earlier and represents the applicant(s) also for international preliminary examination.

☐ is hereby appointed and any earlier appointment of (an) agent(s)/common representative is hereby revoked.

☐ is hereby appointed, specifically for the procedure before the International Preliminary Examining Authority, in addition to the agent(s)/common representative appointed earlier.

Name and address:	Telephone No.
MCLAUGHLIN, Michael Gerard **McLaughlin IP Pte. Ltd.** **24A Mosque Street** **Singapore 059504** **Singapore**	64482168
	Facsimile No.
	Agent's registration No. with the Office

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☑ as advance copies followed by paper notifications; or ☐ exclusively in electronic form (no paper notifications will be sent).

E-mail address: mail@mclaughlinip.com

☐ **Address for correspondence:** Mark this check-box where no agent or common representative is/has been appointed and the space above is used instead to indicate a special address to which correspondence should be sent.

Box No. IV BASIS FOR INTERNATIONAL PRELIMINARY EXAMINATION

Statement concerning amendments:*

1. The applicant wishes the international preliminary examination to start on the basis of:

   the description
   ☑ as originally filed, or
   ☐ as amended under Article 34

   the sequence listing (if any)
   ☐ as originally filed, or
   ☐ as amended under Article 34
   ☐ in the form of an Annex C/ST.25 text file
   ☐ in the form of an image file

   the claims
   ☐ as originally filed, or
   ☐ as amended under Article 19, and/or
   ☑ as amended under Article 34

   the drawings
   ☐ as originally filed, or
   ☑ as amended under Article 34

2. ☐ The applicant wishes any amendment to the claims under Article 19 to be considered as reversed.
3. ☐ Where the IPEA wishes to start the international preliminary examination at the same time as the international search in accordance with Rule 69.1(b), the applicant requests the IPEA **to postpone** the start of the international preliminary examination until the expiration of the applicable time limit under Rule 69.1(d).
4. ☑ The applicant expressly wishes the international preliminary examination **to start earlier** than at the expiration of the applicable time limit under Rule 54bis.1(a).

* Where no check-box is marked, international preliminary examination will start on the basis of the international application as

originally filed or, where a copy of amendments to the claims under Article 19 and/or amendments of the international application under Article 34 are received by the International Preliminary Examining Authority before it has begun to draw up a written opinion or the international preliminary examination report, as so amended.

Language for the purposes of international preliminary examination: English

☑ which is the language in which the international application was filed.

☐ which is the language of a translation furnished for the purposes of international search.

☐ which is the language of publication of the international application.

☐ which is the language of the translation (to be) furnished for the purposes of international preliminary examination.

Box No. V ELECTION OF STATES

The filing of this demand constitutes the election of all Contracting States which are designated and are bound by Chapter II of the PCT.

Box No. VI CHECK LIST

The demand is accompanied by the following elements, in the language referred to in Box No. IV, for the purposes of international preliminary examination:

- Letter accompanying amendments under Article 34: 1 document
- Article 34 Amendments: 4 documents

Box No. VII SIGNATURE OF APPLICANT, AGENT OR COMMON REPRESENTATIVE

Next to each signature, indicate the name of the person signing and the capacity in which the person signs (if such capacity is not obvious from reading the demand).

/MICHAEL GERARD MCLAUGHLIN//

Name (LAST, First): MCLAUGHLIN, Michael Gerard

For International Preliminary Examining Authority use only

1. Date of actual receipt of DEMAND: 26 March 2018 (26.03.2018)	
2. Adjusted date of receipt of demand due to CORRECTIONS under Rule 60.1(b):	
3. ☐ The date of receipt of the demand is AFTER the expiration of 19 months from the priority date and item 4 or 5, below, does not apply. ☐ The applicant has been informed accordingly. 4. ☐ The date of receipt of the demand is WITHIN the time limit of 19 months from the priority date as extended by virtue of Rule 80.5. 5. ☐ Although the date of receipt of the demand is after the expiration of 19 months from the priority date, the delay in arrival is EXCUSED pursuant to Rules 82 or 82quater .	6. ☐ The date of receipt of the demand is AFTER the expiration of the time limit under Rule 54bis.1(a) and item 7 or 8, below, does not apply. 7. ☐ The date of receipt of the demand is WITHIN the time limit under Rule 54bis.1(a) as extended by virtue of Rule 80.5. 8. ☐ Although the date of receipt of the demand is after the expiration of the time limit under Rule 54bis.1(a), the delay in arrival is EXCUSED pursuant to Rules 82 or 82quater.

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Demand received from IPEA on:

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FEE CALCULATION SHEET

Annex to the Demand

International application No. PCT/SG2017/050500	**For International Preliminary Examining Authority use only**
Applicant's or agent's file reference P1007-WO	Date stamp of the IPEA

Applicant

CELLRESEARCH CORPORATION PTE. LTD.

CALCULATION OF PRESCRIBED FEES

1. Preliminary examination fee	830.0 (SGD) P
2. Handling fee (Applicants from certain States are entitled to a reduction of 90% of the handling fee. Where the applicant is (or all applicants are) so entitled, the amount to be entered at H is 10% of the handling fee.)	280.0 (SGD) H
3. Total of prescribed fees Add the amounts entered at P and H and enter total in the TOTAL box	1110.00 (SGD) TOTAL

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Signature:

McLaughlin IP

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REGISTERED DESIGN ATTORNEYS

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Singapore 189554

Our Ref: P1007-WO/EL/jg
Your Ref: PCT/SG2017/050500

Date: 26 March 2018

Attn: Dr. Chen Xiuli

International (PCT) Patent Application PCT/SG2017/050500
Applicant: CELLRESEARCH CORPORATION PTE LTD
Title: A METHOD OF ISOLATING MESENCHYMAL STEM CELLS FROM THE AMNIOTIC MEMBRANE OF THE UMBILICAL CORD, A MESENCHYMAL STEM CELL POPULATION ISOLATED FROM THE AMNIOTIC MEMBRANE OF THE UMBILICAL CORD AND A CELL CULTURE MEDIUM FOR ISOLATING MESENCHYMAL STEM CELLS FROM THE AMNIOTIC MEMBRANE OF THE UMBILICAL CORD

In response to the Written Opinion of the International Searching Authority pursuant to PCT Rule 43bis.1 dated 31 January 2018:

1. Filed documents/Requests

On behalf of the Applicant, we file herewith a Demand for International Preliminary Examination (IPE) of this International Patent Application and it is requested to start the examination on the basis of claims 1 to 60 filed herewith. In order to facilitate the work of the Examiner, an annotated copy of the claims in which changes made to the original set of claims is also filed herewith. In addition, amended drawing sheets 11/12 and 12/12 are filed herewith.

For the event that the Examiner may still have reservations concerning the patentability of the claimed subject matter, we request a Written Opinion of the IPEA under Rule 66.2 PCT prior to issuance of the IPRP Chapter II.

2. Amended drawing sheets

Drawing sheet 11/12 has been amended by adding its figure numbering (Fig. 7a) which was inadvertently missing on the drawing sheet as original filed. Support for this amendment (of an obvious error) can be found in the description of Fig. 7a in page 5, lines 30-32 which explains that *"**Fig. 7a** shows the percentage of isolated mesenchymal cord lining stem cells that express*

the stem cell markers CD73, CD90 and CD105 and lack expression of CD34, CD45 and HLA-DR after isolation from umbilical cord tissue and cultivation in PTT-6 medium".

Drawing sheet 12/12 has been amended by adding its figure numbering (Fig. 7b) which was inadvertently missing on the drawing sheet as original filed. Support for this amendment (of an obvious error) can be found in the description of Fig. 7b in page 5, lines 33-34 which explains that *"**Fig. 7b** shows the percentage of isolated bone marrow mesenchymal stem cells that express CD73, CD90 and CD105 and lack expression of CD34, CD45 and HLA-DR".*

3. Claim Amendments

The new claims 1 to 60 correspond to original claims 1 to 61 which have been amended as follows (see also the attached annotated copy of the claims).

No changes have been made to claims 1 to 32, apart from correcting the dependency in claim 11.

New claim 33 corresponds to original claim 33 in which it has been defined that at least 97 % or more cells of the isolated mesenchymal stem cell population express each of CD73, CD90 and CD105 and lack expression of each of CD34, CD45 and HLA-DR. This amendment finds support in original claim 35.

Due to the amendment of claim 33, original claim 34 has been deleted.

New claim 34 corresponds to original claim 35 which has been amended in line with the amendment to claim 33 to now recite that at least about 98 % or more about 99 % or more cells of the isolated mesenchymal stem cell population express each of CD73, CD90 and CD105 and lack expression of each of CD34, CD45 and HLA-DR.

New claims 35 and 36 correspond to original claims 36 and 37.

New claim 37 corresponds to original claim 38 which has been amended in line with the amendment to claim 33 to recite that at least 97 % or more cells of the isolated mesenchymal stem cell population express each of CD73, CD90 and CD105 and lack expression of each of CD34, CD45 and HLA-DR.

New claims 38 to 60 correspond to original claims 39 to 61 the numbering of which has been adapted to the new claim numbering.

4. Patentability

In the International Search Report (ISR) the Examiner cites the following eight references in connection with the patentability of the claimed subject matter.

D1: Subramanian et al, PLoS ONE 10(6): e0127992, 2015,
D2: Van Pham et al., Cell Tissue Bank (2016) 17:289-302, 2016,
D3: Vu et al, Biomedical Research and Therapy, 2(2): 207-219, 2015,
D4: WO 2006/019537,
D5: WO 2007/046775,
D6: CN 105420179 and D6*: English machine translation of D6,
D7: Do D.V. et al, Chapter 12 Derivation of Hepatocytes from Umbilical Cord Lining Epithelial Stem Cells, Stem Cells; From Bench to Bedside 2nd Edition, November 2010, pages 323-337, and
D8: Conconi et al, The Open Tissue Engineering and Regenerative Medicine Journal, 2011, 4, 6-20 2011.

In light of these references, the patentability of the subject matter of claims 1 to 32 and 41 to 61 was acknowledged while novelty and/or inventive step was denied for the subject matter of claims 33 to 40 as discussed in the following.

4.1. Novelty

The novelty of the mesenchymal stem cell population of claims 33 to 37 over reference D1 was denied in the Written Opinion. We would like to address this assessment as follows.

As the Examiner indicated in the Written Opinion reference D1 discloses the comparative characterization of mesenchymal stem cells from various compartments of the human umbilical cord, namely of mesenchymal stem cells isolated from the amnion (AM), subamnion (SA), perivascular (PV), Wharton's jelly (WJ) and mixed cord (MC). We note in this context that the subamnion is a different name for the amniotic membrane which is also called the 'cord lining' (see D1, page 2, Introduction, second paragraph or present application, paragraph bridging pages 1 and 2).

With respect to the expression of cell markers D1 discloses the following on page 9 in the section "CD marker analysis":

> *The cell populations derived from all regions of the UC (WJ, PV, SA, AM and MC) were positive for the MSC-CD signature markers CD29, CD44, CD73, CD90, CD105 and HLA-ABC (97.81 ± 0.37% to 99.50 ± 0.11%) and* ***negative for CD14, CD19, CD34, CD45, CD117 and***

HLA-DR ABC (3.73 ± 0.26% to 22.43 ± 0.46%) *with no statistically significant differences in the percentages between the markers for the various regions".* (Emphasis added)

Thus, D1 only discloses a range of the percentages in which the negative markers are expressed by the mesenchymal stem cell populations. D1 does not disclose the individual expression level of each negative marker by each of the characterized stem cell population. In addition, the lowest percentage in which the negative markers are expressed, is 3.73 ± 0.26%, i.e. 3.47% of the cells given the error margin. Put differently, this means that **at best 96.53 %** of the cells of one the mesenchymal stem cell populations lack expression of one of the negative markers CD14, CD19, CD34, CD45, CD117 and HLA-DR ABC.

In contrast, amended claim 33 defines **that at least 97 % or more** cells of the isolated mesenchymal stem cell population express each of CD73, CD90 and CD105 and lack expression of each of CD34, CD45 and HLA-DR. Accordingly, the isolated mesenchymal stem population of the amniotic membrane of the umbilical cord as defined in amended claim 33 is novel over D1.

Since new claims 34 to 36 are dependent on claim 33, the stem cell population of claims 34 to 36 is also novel over D1.

The Examiner is thus kindly requested to acknowledge the novelty for the entire claimed subject matter.

5. Inventive Step

5.1 Claims 33 to 37

5.1.1 Reference D1

In light of the novelty objection to original claims 33 to 37, the Examiner concluded that original claims 33 to 37 do not involve an inventive step over reference D1.

As explained above, the lowest percentage in which the negative markers are expressed by the cells of one of the mesenchymal stem cell populations in D1 is 3.73 ± 0.26%, i.e. 3.47% of the cells given the error margin. Or, as in also said above, this means that at best 96.53 % cells of one of mesenchymal stem cell populations lack expression of one of the six markers CD14, CD19, CD34, CD45, CD117 and HLA-DR ABC. In addition, and even more importantly, D1 describes that the range in which the mesenchymal stem cell populations analysed in D1 express the negative markers is 3.73 ± 0.26% to **22.43± 0.46%**. This means that, given the error margin, only 78.03 % of the cells of one the mesenchymal stem cell populations lack expression of this (unspecified) negative marker. In addition, the remaining other four negative markers are expressed by a percentage of the cells of the respective mesenchymal stem cell population in between 78 %

and at 96%. In addition, D1 does not give any hint or suggestion to improve the purity/homogeneity of the respective mesenchymal stem cell population.

Accordingly, the isolated mesenchymal stem cell population of claim 33 of which at 97 % or more cells express each of CD73, CD90 and CD105 and lack expression of each of CD34, CD45 and HLA-DR is inventive over reference D1.

In this respect, we also refer to the experimental characterization of the cell population of the invention in Example 3 and Figs. 6 and 7 of the PCT application as filed. As explained in lines 25 to 29 of page 34 of the present application, **Fig. 7a** shows that

> *"the mesenchymal stem cell population contained 97.5 % viable cells of which 100 % expressed each of CD73, CD90 and CD105 (see the rows "CD73+CD90+" and "CD73+CD105+") while 99.2 % of the stem cell population did not express CD45 and 100 % of the stem cell population did not express CD34 and HLA-DR (see the rows "CD34-CD45-and "CD34-HLA-DR-)."*

In this context, we refer to the criteria generally accepted for human mesenchymal stem cells to be used for cellular therapy as defined, for example, by Dominici et al, "Minimal criteria for defining multipotent mesenchymal stromal cells. The International Society for Cellular Therapy position statement", Cytotherapy (2006). This publication has been cited in the present application and is submitted herewith as **Annex A** for the ease of reference. As stated in page 316, left hand column and summarized in Table 1 of Dominici et al., ≥ **95% of the MSC population must express CD105, CD73 and CD90**, as measured by flow cytometry and additionally, **these cells must lack expression (≤ 2% positive)** of CD45, CD34, CD14 or CD11b, CD79a or CD19 and HLA class II.

Thus, it is evident that the mesenchymal stem cell population of the present invention meet the criteria generally accepted for human mesenchymal stem cells to be used for cellular therapy while none of the stem cell populations described in D1 do. According, the stem cell population of the present invention being an extremely homogenous and well defined cell population is an ideal candidate for clinical trials and cell based therapies since and therefore provides a significant advantage over the stem cell populations described in reference D1.

The Examiner is therefore respectfully request to acknowledge the existence of an inventive step for the mesenchymal stem cell population of amended independent claim 33 and thus also for the cell population of new claims 34 to 36 over reference D1.

5.1.2 Reference D1

In the Written Opinion inventive step was denied for the subject matter of original claims 33 to 37 also over reference D2. According to the Examiner, D2 discloses an isolated mesenchymal stem cell (MSC) population from the umbilical cord (amniotic membrane) and Wharton's jelly, wherein 90 % or more of the stem cell population in group IV is positive for CD73, CD90, and CD105, while 94 % or more lack expression of CD34, CD45 and HLA-DR (D2: abstract, page 10, left column, Fig. 3 see group IV).

The Examiner states that D2 mainly differs from claims 33-37 in that the isolation of the MSC population is not exclusively from the amniotic membrane. However, in the absence of any unexpected results by using only the amniotic membrane, it is merely a matter of choice to the skilled person. The Examiner concludes that therefore original claims 33 to 37 lack inventive step in view of D2.

Addressing this objection, we copy below the results for the expression of the mesenchymal stem cell markers CD73, CD90, and CD105 (positive markers) and CD34, CD45 and HLA-DR (negative markers) for group IV on which the Examiner has based his assessment.

- CD73: 90.51 ± 3.01 %,
- CD90 : 98.41 ± 2.19 %,
- CD105: 95.21 ± 1.56 %,
- CD34: 4.21 ± 0.92 %,
- CD45: 5.15 ± 1.52 %,
- HLA-DR 4.24 ± 1.14 %.

As can be seen from the results, only 90.51 ± 3.01 % of the cells of the stem cell population of group IV express the positive marker CD73, while more than 3 % of the cells of this population express the three negative markers CD34, CD45 and HLA-DR.

Accordingly, neither does the mesenchymal stem cell population of group IV of D2 meet the definition of the mesenchymal stem cell population of claim 33, nor does this stem cell population meet the requirements for human mesenchymal stem cells to be used for cellular therapy as defined, by Dominici et al.

For the sake of completeness, we add that the percentage of cells of mesenchymal stem cell population of groups I to III and IV of D2 that, for example, express the positive markers CD73, CD90, and CD105 is even lower (expect for the expression of CD105 by Group IV) as shown in the following table.

	Group I	Group II	Group III	Group IV
CD73 expression (%)	86.13 ± 2.09	85.09 ± 4.82	87.75 ± 4.22	88.56 ± 4.18
CD90 expression %)	92.78 ± 2.83	91.01 ± 1.90	93.44 ± 2.12	97.35 ± 2.18
CD105 expression (%)	92.23 ± 3.64	91.42 ± 2.09	93.45 ± 1.22	98.13 ± 1.55

Thus, the stem cell population as defined in amended claim 33 has a technical advantage over the stem cell populations of reference D2.

For this reason, the Examiner is kindly requested to acknowledge the existence of an inventive step for the mesenchymal stem cell population of amended independent claim 33 and thus also for the cell population of claims 34 to 36 also over reference D2.

5.2. Original claims 38 to 40 (new claims 37 to 39)

The Examiner states in the Written Opinion that each of D1 and D2 differs from original claims 38 to 40 in that D1 or D2 is silent about a pharmaceutical composition comprising the isolated MSC population. However, it is well within the purview of the skilled person to derive a pharmaceutical formulation comprising MSC population for known therapeutic purposes. The Examiner thus concludes that the pharmaceutical composition of original claims 38 to 40 lack inventive ingenuity.

As shown above, the mesenchymal stem cell population of new claims 33 to 36 is inventive over both references D1 and D2. Accordingly, also the pharmaceutical composition of new claims 37 to 39 is inventive over references D1 and D2.

As patentability of the subject matter of original claims 1 to 32 and 41 to 61 was already expressly acknowledged the Examiner, the Examiner is respectfully asked to acknowledge that the entire claimed subject matter of new claims 1 to 60 is patentable over the cited references.

6. Conclusion

In light of the above, we look forward to receipt of an International Preliminary Examination Report (IPRP) (Chapter II) which is positive for all claims. Should the Examiner have any further concerns in this respect, issuance of a Written Opinion of the International Preliminary Examining Authority (IPEA) under Rule 66.2 PCT is kindly requested.

McLaughlin

The Examiner is also invited to contact us by telephone or by email should clarification be required on any matter.

Yours faithfully,

Michael McLaughlin
McLaughlin IP Pte. Ltd.

Encls.

- New claims 1 to 60 (clean and marked copy)
- New drawing sheets 1/12 to 12/12 (clean and marked copy)
- Annex A: Dominici et al, "Minimal criteria for defining multipotent mesenchymal stromal cells. The International Society for Cellular Therapy position statement", Cytotherapy (2006).

International (PCT) Patent Application PCT/SG2017/050500

Applicant: CELLRESEARCH CORPORATION PTE LTD

Title: A METHOD OF ISOLATING MESENCHYMAL STEM CELLS FROM THE AMNIOTIC MEMBRANE OF THE UMBILICAL CORD, A MESENCHYMAL STEM CELL POPULATION ISOLATED FROM THE AMNIOTIC MEMBRANE OF THE UMBILICAL CORD AND A CELL CULTURE MEDIUM FOR ISOLATING MESENCHYMAL STEM CELLS FROM THE AMNIOTIC MEMBRANE OF THE UMBILICAL CORD

ANNEX A

International Society for Cellular Therapy ISCT

Cytotherapy (2006) Vol. 8, No. 4, 315–317

Taylor & Francis
Taylor & Francis Group

POSITION PAPER

Minimal criteria for defining multipotent mesenchymal stromal cells. The International Society for Cellular Therapy position statement

M Dominici[1], K Le Blanc[2], I Mueller[3], I Slaper-Cortenbach[4], FC Marini[5], DS Krause[6], RJ Deans[7], A Keating[8], DJ Prockop[9] and EM Horwitz[10]

[1]*Laboratory of Cell Biology and Advanced Cancer Therapy, Oncology-Hematology Department, University of Modena and Reggio Emilia, Modena, Italy,* [2]*Center for Allogeneic Stem Cell Transplantation, Department of Laboratory Medicine, Karolinska University Hospital, Karolinska Institute, Stockholm, Sweden,* [3]*University Children's Hospital, Department of Hematology and Oncology, Tuebingen, Germany,* [4]*Department of Medical Immunology, UMC Utrecht, Utrecht, the Netherlands,* [5]*Department of Blood and Marrow Transplant, UT-MD Anderson Cancer Center, Houston, Texas, USA,* [6]*Department of Laboratory Medicine, Yale University School of Medicine, New Haven, Connecticut, USA,* [7]*Athersys Inc., Cleveland, Ohio, USA,* [8]*Department of Medical Oncology and Hematology Princess Margaret Hospital/Ontario Cancer Institute Toronto, Ontario, Canada,* [9]*Center for Gene Therapy, Tulane University Health Sciences Center, New Orleans, Louisiana, USA and* [10]*Divisions of Stem Cell Transplantation and Experimental Hematology, St Jude Children's Research Hospital, Memphis Tennessee, USA*

The considerable therapeutic potential of human multipotent mesenchymal stromal cells (MSC) has generated markedly increasing interest in a wide variety of biomedical disciplines. However, investigators report studies of MSC using different methods of isolation and expansion, and different approaches to characterizing the cells. Thus it is increasingly difficult to compare and contrast study outcomes, which hinders progress in the field. To begin to address this issue, the Mesenchymal and Tissue Stem Cell Committee of the International Society for Cellular Therapy proposes minimal criteria to define human MSC. First, MSC must be plastic-adherent when maintained in standard culture conditions. Second, MSC must express CD105, CD73 and CD90, and lack expression of CD45, CD34, CD14 or CD11b, CD79α or CD19 and HLA-DR surface molecules. Third, MSC must differentiate to osteoblasts, adipocytes and chondroblasts in vitro. *While these criteria will probably require modification as new knowledge unfolds, we believe this minimal set of standard criteria will foster a more uniform characterization of MSC and facilitate the exchange of data among investigators.*

Keywords

MSC, stem cells, adherent cells, immunophenotype, differentiation.

Biologic and clinical interest in MSC has risen dramatically over the last two decades, as shown by the ever-increasing number of research teams studying these cells. Not only are established laboratories focusing on MSC but new investigators are rapidly being attracted to the field, which will undoubtedly accelerate scientific discovery and the development of novel cellular therapies. However, this soaring interest has also generated many ambiguities and inconsistencies in the field.

To begin to address these issues, a recent report from the International Society for Cellular Therapy (ISCT) stated that 'multipotent mesenchymal stromal cells' (MSC) is the currently recommended designation [1] for the plastic-adherent cells isolated from BM and other tissues that have often been labeled mesenchymal stem cells [2].

The defining characteristics of MSC are inconsistent among investigators. Many laboratories have developed methods to isolate and expand MSC, which invariably have subtle, and occasionally quite significant, differences. Furthermore, investigators have isolated MSC from a variety of tissues with ostensibly similar properties [3]. These varied tissue sources and methodologies of cell

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DOI: 10.1080/14653240600855905

preparation beg the question of whether the resulting cells are sufficiently similar to allow for a direct comparison of reported biologic properties and experimental outcomes, especially in the context of cell therapy. This question of cell equivalence is, in part, because of the lack of universally accepted criteria to define MSC. Most importantly, the inability to compare and contrast studies from different groups is likely to hinder progress in the field.

To address this problem, the Mesenchymal and Tissue Stem Cell Committee of the ISCT proposes a set of standards to define human MSC for both laboratory-based scientific investigations and for pre-clinical studies. These identifying criteria should not be confused with release specifications for clinical studies, as the current proposal is intended solely as identifying criteria for research purposes. The aim of this position statement is to provide the scientific community with a standard set of criteria, based on the best currently available data, to define the identity of MSC, recognizing that future research will probably mandate a revision of the criteria as new data emerge.

We propose three criteria to define MSC:

- adherence to plastic
- specific surface antigen (Ag) expression
- multipotent differentiation potential (Table 1).

First, MSC must be plastic-adherent when maintained in standard culture conditions using tissue culture flasks. Second, $\geq 95\%$ of the MSC population must express CD105, CD73 and CD90, as measured by flow cytometry. Additionally, these cells must lack expression ($\leq 2\%$ positive) of CD45, CD34, CD14 or CD11b, CD79α or CD19 and HLA class II. Third, the cells must be able to differentiate to osteoblasts, adipocytes and chondroblasts under standard *in vitro* differentiating conditions.

Table 1. Summary of criteria to identify MSC

1	Adherence to plastic in standard culture conditions		
2	Phenotype	Positive ($\geq 95\%+$)	Negative ($\leq 2\%+$)
		CD105	CD45
		CD73	CD34
		CD90	CD14 or CD11b
			CD79α or CD19
			HLA-DR
3	*In vitro* differentiation: osteoblasts, adipocytes, chondroblasts (demonstrated by staining of *in vitro* cell culture)		

Plastic adherence is a well-described property of MSC, and even unique subsets of MSC that have been described maintain this property [4,5]. While MSC may be maintained, and possibly expanded, without adherence [6], these protocols typically require very specific culture conditions, and these cells, if maintained under more standard conditions, would be expected to demonstrate adherence if the cells are to be considered a population of MSC.

Surface Ag expression, which allows for a rapid identification of a cell population, has been used extensively in immunology and hematology. To identify MSC, we propose that cells should express CD105 (known as endoglin and originally recognized by the MAb SH2), CD73 (known as ecto 5′ nucleotidase and originally recognized by the MAb SH3 and SH4) and CD90 (also known as Thy-1). Novel surface markers that may be identified in the future could lead to modifications of these criteria. To assure that studies of heterogeneous populations of MSC are not confounded by other cells, we recommend that lack of expression of hematopoietic Ag be used as additional criteria for MSC as they are not known to express these Ag. For this purpose, we recommend that a panel of Ag be used to exclude the cells most likely to be found in MSC cultures. CD45 is a pan-leukocyte marker; CD34 marks primitive hematopoietic progenitors and endothelial cells; CD14 and CD11b are prominently expressed on monocytes and macrophages, the most likely hematopoietic cells to be found in an MSC culture; CD79α and CD19 are markers of B cells that may also adhere to MSC in culture and remain vital through stromal interactions; and HLA-DR molecules are not expressed on MSC unless stimulated, e.g. by IFN-γ. Only one of the two macrophage and B-cell markers needs to be tested. Each group of investigators should select the marker(s) that is (are) most reliable in their laboratory.

Finally, the biologic property that most uniquely identifies MSC is their capacity for trilineage mesenchymal differentiation. Thus, cells must be shown to differentiate to osteoblasts, adipocytes and chondroblasts using standard *in vitro* tissue culture-differentiating conditions. Differentiation to osteoblasts can be demonstrated by staining with Alizarin Red or von Kossa staining. Adipocyte differentiation is most readily demonstrated by staining with Oil Red O. Chondroblast differentiation is demonstrated by staining with Alcian blue or immunohis-

tochemical staining for collagen type II. Most published protocols for such differentiations are similar, and kits for such assays are now commercially available; thus, we believe that demonstrating differentiation should be feasible for all investigators.

Several of the criteria merit further comment. First, we encourage investigators to test for as many surface markers (both positive and negative) as they deem important, especially as it relates to their own research. The optimum flow cytometric assay would utilize multicolor analyzes (i.e. double staining, triple staining, etc.) to demonstrate that individual cells co-express MSC markers and lack hematopoietic Ag. Our proposal represents the minimum requirements, but additional evidence is always useful.

We also recognize that the proposed panel of Ag does not uniquely identify MSC compared with some other cells [7]. However, the surface phenotype, in conjunction with the other functional criteria, best identifies MSC with the current state of knowledge.

Second, MSC express HLA-DR surface molecules in the presence of IFN-γ but not in an unstimulated state. Thus, if HLA-DR expression is found, and in fact such expression may be desirable for some applications, the cells may still be termed MSC, assuming the other criteria are met, but should be qualified with adjectives, such as 'stimulated MSC', or other nomenclature to indicate that the cells are not in the baseline state.

Third, the level of MSC purity we suggest ($\geq 95\%$ expression of CD105, CD73, CD90; $\leq 2\%$ expression of hematopoietic Ag) should be considered as minimal guidelines. Greater levels of demonstrated purity may be required for some experimental systems.

Finally, MSC have great propensity for *ex vivo* expansion. Investigators who utilize extensively passaged cells may be well served by verifying a normal karyotype to reduce the probability of chromosomal abnormalities, including potentially transforming events. Such events could potentially lead to the establishment of a novel cell line, and the resulting cells should no longer be considered MSC. However, karyotype analysis is not being recommended for routine identification of MSC.

These criteria apply only to human MSC. While adherence and trilineage differentiation are characteristics of cells from other species, for example murine MSC, surface Ag expression is not universally well characterized [8] and the Ag recommended may not apply to non-human systems. Moreover, these criteria should be employed in a control study fashion. Investigators should demonstrate that the cells prepared in their laboratory for a given project meet the stated criteria; however, each cell preparation does not need to be re-evaluated.

Our goal is to encourage MSC investigators to adopt these minimal criteria in an effort to standardize the cell preparations and allow for a comparison of scientific studies among laboratories. These criteria should become the basis for additional characterization of these cells. By this approach, we anticipate more rapid advances in the use of these cells in pre-clinical studies and in the subsequent development of clinical therapies.

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What is claimed is:

1. A method of isolating a mesenchymal stem cell population from the amniotic membrane of the umbilical cord, the method comprising cultivating umbilical cord tissue in a culture medium comprising DMEM (Dulbecco's modified eagle medium), F12 (Ham's F12 Medium), M171 (Medium 171) and FBS (Fetal Bovine Serum).

2. The method of claim 1, wherein the culture medium comprises DMEM in a final concentration of about 55 to 65 % (v/v), F12 in a final concentration of about 5 to 15 % (v/v) , M171 in a final concentration of about 15 to 30 % (v/v) and FBS in a final concentration of about 1 to 8 % (v/v).

3. The method of claim 2, wherein the culture medium comprises DMEM in a final concentration of about 57.5 to 62.5 % (v/v), F12 in a final concentration of about 7.5 to 12.5 % (v/v) , M171 in a final concentration of about 17.5 to 25.0 % (v/v) and FBS in a final concentration of about 1.75 to 3.5 % (v/v).

4. The method of claim 3, wherein the culture medium comprises DMEM in a final concentration of about 61.8 % (v/v), F12 in a final concentration of about 11.8 % (v/v) , M171 in a final concentration of about 23.6 % (v/v) and FBS in a final concentration of about 2.5 % (v/v).

5. The method of any of claims 1 to 4, wherein the culture medium further comprises Epidermal Growth Factor (EGF) in a final concentration of about 1 ng/ml to about 20 ng/ml.

6. The method of any of claims 1 to 5, wherein the culture medium comprises EGF in a final concentration of about 10ng/ml.

7. The method of any of claims 1 to 6, wherein the culture medium comprises Insulin in a final concentration of about 1 µg/ml to 10 µg/ml.

8. The method of any of claims 1 to 7, wherein the culture medium comprises Insulin in a final concentration of about 5µg/ml.

9. The method of any of the foregoing claims, wherein the culture medium further comprises at least one of the following supplements: adenine, hydrocortisone, and 3,3′,5-Triiodo-L-thyronine sodium salt (T3).

10. The method of any of the foregoing claims, wherein the culture medium comprises all three of adenine, hydrocortisone, and 3,3′,5-Triiodo-L-thyronine sodium salt (T3).

11. The method of claim ~~11 or 12~~10, wherein the culture medium comprises adenine in a final concentration of about 0.01 to about 0.1 µg/ml adenine, hydrocortisone in a final concentration of about 0.1 to about 10 µg/ml hydrocortisone and/or 3,3′,5-Triiodo-L-thyronine sodium salt (T3) in a final concentration of about 0.5 to about 5 ng/ml.

12. The method of any of the foregoing claims, comprising culturing the umbilical cord tissue till the cell outgrowth of the mesenchymal stem cells of the amniotic membrane reaches about 70-80% confluency.

13. The method of claim 12, comprising removing the mesenchymal stem cells from the cultivation container used for the cultivation.

14. The method of claim 13, wherein removing the mesenchymal stem cells from the cultivation container is carried out by enzymatic treatment.

15. The method of claim 14, wherein the enzymatic treatment comprises trypsination.

16. The method of any of claims 13 to 15, wherein the mesenchymal stem cells are transferred for subculturing to a cultivation container for subculturing.

17. The method of claim 16, wherein the mesenchymal cells are suspended for subculturing at a concentration 1.0 x 10^6 cells/ml.

18. The method of claim 17, wherein the mesenchymal stem cells are subcultured in a culture medium as defined in any of the claims 1 to 10.

19. The method of claim 18, wherein the mesenchymal stem cells are subcultured till the mesenchymal stem cells reach about 70-80% confluency.

20. The method of any of claims 16 to 19, wherein the subculturing is carried out in a self-contained bioreactor.

21. The method of claim 20, wherein the bioreactor is selected from the group consisting of a parallel-plate bioreactor, a hollow-fiber bioreactor and and a micro-fluidic bioreactor.

22. The method of any of the foregoing claims, wherein the umbilical cord tissue is a piece of the entire umbilical cord or the amniotic membrane of the umbilical cord.

23. The method of any of the foregoing claims wherein cultivation is carried out in a CO_2 cell culture incubator at a temperature 37°C.

24. The method of claim 23, comprising removing the mesenchymal stem cells from the cultivation container used for the subcultivation.

25. The method of claim 24, wherein removing the mesenchymal stem cells from the cultivation container is carried out by enzymatic treatment.

26. The method of claim 25, wherein the enzymatic treatment comprises trypsination.

27. The method of claim 26, further comprising collecting the isolated mesenchymal stem cells.

28. The method of any of the foregoing claims, wherein at least about 90 % or more, about 91 % or more, about 92 % or more, about 92 % or more, about 93 % or more, about 94 % or more, about 95 % or more, about 96 % or more, about 97 % or more, about 98 % or more about 99 % or more of the isolated mesenchymal stem cells express the following markers: CD73, CD90 and CD105.

29. The method of any of the foregoing claims, wherein at least about 90 % or more, about 91 % or more, about 92 % or more, about 92 % or more, about 93 % or more, about 94 % or more, about 95 % or more, about 96 % or more, about 97 % or more, about 98 % or more about 99 % or more of the isolated mesenchymal stem cells lack expression of the following markers: CD34, CD45 and HLA-DR (Human Leukocyte Antigen – antigen D Related).

30. The method of any of claims 28 or 29, wherein about 97 % or more, about 98 % or more about 99 % or more of the isolated mesenchymal stem cells express CD73, CD90 and CD105 and lack expression of CD34, CD45 and HLA-DR.

31. The method of any of the foregoing claims, further comprising preserving the isolated stem/progenitor cells for further use.

32. The method of claim 31, wherein preserving is carried out by cryo-preservation.

33. An isolated mesenchymal stem population of the amniotic membrane of the umbilical cord, wherein at least 97 % or more cells of the isolated mesenchymal stem cell population express each of CD73, CD90 and CD105 and lack expression of each of CD34, CD45 and HLA-DR. ~~at least about 90 % or more cells of the stem cell population express each of the following markers: CD73, CD90 and CD105.~~

~~34. The mesenchymal stem cell population of claim 33, wherein least about 90 % or more cells of the stem cell population lack expression of the following markers: CD34, CD45 and HLA-DR.~~

345. The mesenchymal stem cell population of claim 334, wherein at least ~~about 91 % or more, about 92 % or more, about 92 % or more, about 93 % or more, about 94 % or more, about 95 % or more, about 96 % or more, about 97 % or more,~~ about 98 % or more about 99 % or more cells of the isolated mesenchymal stem cell population express each of CD73, CD90 and CD105 and lack expression of each of CD34, CD45 and HLA-DR.

356. The mesenchymal stem cell population of any of claims 33 to 345, wherein the population is obtainable by the method as defined in any of claims 1 to 30.

367. The mesenchymal stem cell population of any of claims 33 to 345, wherein the population is obtained by the method as defined in any of claims 1 to 30.

378. A pharmaceutical composition comprising an isolated mesenchymal stem population of the amniotic membrane of the umbilical cord, wherein at least ~~about~~ 970 % or more cells of the stem cell population express each of the following markers: CD73, CD90 and CD105 and lack expression of each of the following markers: CD34, CD45 and HLA-DR.

389. The pharmaceutical composition of claim 378, wherein the pharmaceutical composition is adapted for systemic or topical application.

3940. The pharmaceutical composition of claim 378 or 389, further comprising a pharmaceutically acceptable excipient.

401. A method of making a culture medium suitable for isolating a mesenchymal stem cell population from the amniotic membrane of the umbilical cord, the method comprising, mixing to obtain a final volume of 500 ml culture medium:

i. 250 ml of DMEM

ii. 118 ml M171

iii. 118 ml DMEM/F12

iv. 12.5 ml Fetal Bovine Serum (FBS) (final concentration of 2.5%)

412. The method of claim 401, further comprising adding

v. 1 ml EGF stock solution (5 µg/ml) to achieve a final concentration of 10ng/ml)

vi. Insulin 0.175 ml stock solution (14.28 mg/ml) to achieve a final concentration of 5µg/ml.

423. The method of claim 401 or 412, further comprising adding to DMEM one or more of the following supplements: adenine, hydrocortisone, 3,3′,5-Triiodo-L-thyronine sodium salt (T3), thereby reaching a total volume of 500 ml culture medium.

434. The method of claim 413, wherein the final concentration of the supplements in DMEM are as follows.

about 0.05 to 0.1 µg/ml adenine, for example about 0.025 µg/ml adenine,

about 1 to 10 µg/ml hydrocortisone,

about 0.5 to 5 ng/ml 3,3′,5-Triiodo-L-thyronine sodium salt (T3), for example 1.36 ng/ml 3,3′,5-Triiodo-L-thyronine sodium salt (T3).

445. A cell culture medium obtainable by the method of any of claims 401 to 434.

456. A method of isolating mesenchymal stem cells from the amniotic membrane of the umbilical cord, comprising cultivating amniotic membrane tissue in the culture medium prepared by the method as defined in any of claims 401 to 434.

467. A cell culture medium comprising:

- DMEM in the final concentration of about 55 to 65 % (v/v),

- F12 in a final concentration of about 5 to 15 % (v/v),

- M171 in a final concentration of about 15 to 30 % (v/v) and

- FBS in a final concentration of about 1 to 8 % (v/v).

478. The cell culture medium of claim 467, wherein the culture medium comprises DMEM in the final concentration of about 57.5 to 62.5 % (v/v), F12 in a final concentration of about 7.5 to 12.5 % (v/v), M171 in a final concentration of about 17.5 to 25.0 % (v/v) and FBS in a final concentration of about 1.75 to 3.5 % (v/v).

489. The cell culture medium of claim 478, wherein the culture medium comprises DMEM in a final concentration of about 61.8 % (v/v), F12 in a final concentration of about 11.8 % (v/v), M171 in a final concentration of about 23.6 % (v/v) and FBS in a final concentration of about 2.5 % (v/v).

4950. The cell culture medium of any of claims 467 to 489, wherein the culture medium further comprises Epidermal Growth Factor (EGF) in a final concentration of about 1 ng/ml to about 20 ng/ml.

504. The cell culture medium of any of claims 467 to 4950, wherein the culture medium comprise EGF in a final concentration of about 10ng/ml.

512. The cell culture medium of any of claims 467 to 504, wherein the culture medium comprises Insulin in a final concentration of about 1 µg/ml to 10 µg/ml.

523. The cell culture medium of claim 512, wherein the culture medium comprises Insulin in a final concentration of about 5µg/ml.

534. The cell culture medium of any of claims 467 to 523, wherein the culture medium further comprises at least one of the following supplements: adenine, hydrocortisone, and 3,3′,5-Triiodo-L-thyronine sodium salt (T3).

545. The cell culture medium of claim 534, wherein the culture medium comprises all three of adenine, hydrocortisone, and 3,3′,5-Triiodo-L-thyronine sodium salt (T3).

556. The cell culture medium of claim 534 or 545, wherein the culture medium comprises adenine in a final concentration of about 0.05 to about 0.1 µg/ml adenine, hydrocortisone in a final concentration of about 1 to about 10 µg/ml hydrocortisone and/or 3,3′,5-Triiodo-L-thyronine sodium salt (T3) in a final concentration of about 0.5 to about 5 ng/ml.

567. The cell culture medium of any of claims 467 to 556, wherein 500 ml of the cell culture medium comprise:

i. 250 ml of DMEM

ii. 118 ml M171

iii. 118 ml DMEM/F12

iv. 12.5 ml Fetal Bovine Serum (FBS) (final concentration of 2.5%)

578. The cell culture medium of claim 567, further comprising

v. EGF in a final concentration of 10ng/ml

vi. Insulin in a final concentration of 5µg/ml.

vi. Insulin 0.175 ml (final concentration of 5µg/ml)

589. The cell culture medium of claim 567 or 578, further comprising adenine in a final concentration of about 0.05 to about 0.1 µg/ml adenine, hydrocortisone in a final concentration of about 1 to about 10 µg/ml hydrocortisone and/or 3,3',5-Triiodo-L-thyronine sodium salt (T3) in a final concentration of about 0.5 to about 5 ng/ml.

5960. The use of a cell culture medium as defined in any of claims 467 to 589 for isolation of mesenchymal stem cells from the amniotic membrane of umbilical cord.

601. The use of a cell culture medium as defined in any of claims 467 to 589 for cultivation of mesenchymal stem cells from the amniotic membrane of umbilical cord.

What is claimed is:

1. A method of isolating a mesenchymal stem cell population from the amniotic membrane of the umbilical cord, the method comprising cultivating umbilical cord tissue in a culture medium comprising DMEM (Dulbecco's modified eagle medium), F12 (Ham's F12 Medium), M171 (Medium 171) and FBS (Fetal Bovine Serum).

2. The method of claim 1, wherein the culture medium comprises DMEM in a final concentration of about 55 to 65 % (v/v), F12 in a final concentration of about 5 to 15 % (v/v) , M171 in a final concentration of about 15 to 30 % (v/v) and FBS in a final concentration of about 1 to 8 % (v/v).

3. The method of claim 2, wherein the culture medium comprises DMEM in a final concentration of about 57.5 to 62.5 % (v/v), F12 in a final concentration of about 7.5 to 12.5 % (v/v) , M171 in a final concentration of about 17.5 to 25.0 % (v/v) and FBS in a final concentration of about 1.75 to 3.5 % (v/v).

4. The method of claim 3, wherein the culture medium comprises DMEM in a final concentration of about 61.8 % (v/v), F12 in a final concentration of about 11.8 % (v/v) , M171 in a final concentration of about 23.6 % (v/v) and FBS in a final concentration of about 2.5 % (v/v).

5. The method of any of claims 1 to 4, wherein the culture medium further comprises Epidermal Growth Factor (EGF) in a final concentration of about 1 ng/ml to about 20 ng/ml.

6. The method of any of claims 1 to 5, wherein the culture medium comprises EGF in a final concentration of about 10ng/ml.

7. The method of any of claims 1 to 6, wherein the culture medium comprises Insulin in a final concentration of about 1 µg/ml to 10 µg/ml.

8. The method of any of claims 1 to 7, wherein the culture medium comprises Insulin in a final concentration of about 5µg/ml.

9. The method of any of the foregoing claims, wherein the culture medium further comprises at least one of the following supplements: adenine, hydrocortisone, and 3,3′,5-Triiodo-L-thyronine sodium salt (T3).

10. The method of any of the foregoing claims, wherein the culture medium comprises all three of adenine, hydrocortisone, and 3,3′,5-Triiodo-L-thyronine sodium salt (T3).

11. The method of claim 10, wherein the culture medium comprises adenine in a final concentration of about 0.01 to about 0.1 µg/ml adenine, hydrocortisone in a final concentration of about 0.1 to about 10 µg/ml hydrocortisone and/or 3,3′,5-Triiodo-L-thyronine sodium salt (T3) in a final concentration of about 0.5 to about 5 ng/ml.

12. The method of any of the foregoing claims, comprising culturing the umbilical cord tissue till the cell outgrowth of the mesenchymal stem cells of the amniotic membrane reaches about 70-80% confluency.

13. The method of claim 12, comprising removing the mesenchymal stem cells from the cultivation container used for the cultivation.

14. The method of claim 13, wherein removing the mesenchymal stem cells from the cultivation container is carried out by enzymatic treatment.

15. The method of claim 14, wherein the enzymatic treatment comprises trypsination.

16. The method of any of claims 13 to 15, wherein the mesenchymal stem cells are transferred for subculturing to a cultivation container for subculturing.

17. The method of claim 16, wherein the mesenchymal cells are suspended for subculturing at a concentration 1.0 x 10^6 cells/ml.

18. The method of claim 17, wherein the mesenchymal stem cells are subcultured in a culture medium as defined in any of the claims 1 to 10.

19. The method of claim 18, wherein the mesenchymal stem cells are subcultured till the mesenchymal stem cells reach about 70-80% confluency.

20. The method of any of claims 16 to 19, wherein the subculturing is carried out in a self-contained bioreactor.

21. The method of claim 20, wherein the bioreactor is selected from the group consisting of a parallel-plate bioreactor, a hollow-fiber bioreactor and and a micro-fluidic bioreactor.

22. The method of any of the foregoing claims, wherein the umbilical cord tissue is a piece of the entire umbilical cord or the amniotic membrane of the umbilical cord.

23. The method of any of the foregoing claims wherein cultivation is carried out in a CO_2 cell culture incubator at a temperature 37°C.

24. The method of claim 23, comprising removing the mesenchymal stem cells from the cultivation container used for the subcultivation.

25. The method of claim 24, wherein removing the mesenchymal stem cells from the cultivation container is carried out by enzymatic treatment.

26. The method of claim 25, wherein the enzymatic treatment comprises trypsination.

27. The method of claim 26, further comprising collecting the isolated mesenchymal stem cells.

28. The method of any of the foregoing claims, wherein at least about 90 % or more, about 91 % or more, about 92 % or more, about 92 % or more, about 93 % or more, about 94 % or more, about 95 % or more, about 96 % or more, about 97 % or more, about 98 % or more about 99 % or more of the isolated mesenchymal stem cells express the following markers: CD73, CD90 and CD105.

29. The method of any of the foregoing claims, wherein at least about 90 % or more, about 91 % or more, about 92 % or more, about 92 % or more, about 93 % or more, about 94 % or more, about 95 % or more, about 96 % or more, about 97 % or more, about 98 % or more about 99 % or more of the isolated mesenchymal stem cells lack expression of the following markers: CD34, CD45 and HLA-DR (Human Leukocyte Antigen – antigen D Related).

30. The method of any of claims 28 or 29, wherein about 97 % or more, about 98 % or more about 99 % or more of the isolated mesenchymal stem cells express CD73, CD90 and CD105 and lack expression of CD34, CD45 and HLA-DR.

31. The method of any of the foregoing claims, further comprising preserving the isolated stem/progenitor cells for further use.

32. The method of claim 31, wherein preserving is carried out by cryo-preservation.

33. An isolated mesenchymal stem population of the amniotic membrane of the umbilical cord, wherein at least 97 % or more cells of the isolated mesenchymal stem cell population express each of CD73, CD90 and CD105 and lack expression of each of CD34, CD45 and HLA-DR.

34. The mesenchymal stem cell population of claim 33, wherein at least about 98 % or more about 99 % or more cells of the isolated mesenchymal stem cell population express each of CD73, CD90 and CD105 and lack expression of each of CD34, CD45 and HLA-DR.

35. The mesenchymal stem cell population of any of claims 33 to 34, wherein the population is obtainable by the method as defined in any of claims 1 to 30.

36. The mesenchymal stem cell population of any of claims 33 to 34, wherein the population is obtained by the method as defined in any of claims 1 to 30.

37. A pharmaceutical composition comprising an isolated mesenchymal stem population of the amniotic membrane of the umbilical cord, wherein at least 97 % or more cells of the stem cell population express each of the following markers: CD73, CD90 and CD105 and lack expression of each of the following markers: CD34, CD45 and HLA-DR.

38. The pharmaceutical composition of claim 37, wherein the pharmaceutical composition is adapted for systemic or topical application.

39. The pharmaceutical composition of claim 37 or 38, further comprising a pharmaceutically acceptable excipient.

40. A method of making a culture medium suitable for isolating a mesenchymal stem cell population from the amniotic membrane of the umbilical cord, the method comprising mixing to obtain a final volume of 500 ml culture medium:

i. 250 ml of DMEM

ii. 118 ml M171

iii. 118 ml DMEM/F12

iv. 12.5 ml Fetal Bovine Serum (FBS) (final concentration of 2.5%)

41. The method of claim 40, further comprising adding

v. 1 ml EGF stock solution (5 µg/ml) to achieve a final concentration of 10ng/ml)

vi. Insulin 0.175 ml stock solution (14.28 mg/ml) to achieve a final concentration of 5µg/ml.

42. The method of claim 40 or 41, further comprising adding to DMEM one or more of the following supplements: adenine, hydrocortisone, 3,3′,5-Triiodo-L-thyronine sodium salt (T3), thereby reaching a total volume of 500 ml culture medium.

43. The method of claim 41, wherein the final concentration of the supplements in DMEM are as follows.

about 0.05 to 0.1 µg/ml adenine, for example about 0.025 µg/ml adenine,

about 1 to 10 µg/ml hydrocortisone,

about 0.5 to 5 ng/ml 3,3′,5-Triiodo-L-thyronine sodium salt (T3), for example 1.36 ng/ml 3,3′,5-Triiodo-L-thyronine sodium salt (T3).

44. A cell culture medium obtainable by the method of any of claims 40 to 43.

45. A method of isolating mesenchymal stem cells from the amniotic membrane of the umbilical cord, comprising cultivating amniotic membrane tissue in the culture medium prepared by the method as defined in any of claims 40 to 43.

46. A cell culture medium comprising:

- DMEM in the final concentration of about 55 to 65 % (v/v),

- F12 in a final concentration of about 5 to 15 % (v/v),

- M171 in a final concentration of about 15 to 30 % (v/v) and

- FBS in a final concentration of about 1 to 8 % (v/v).

47. The cell culture medium of claim 46, wherein the culture medium comprises DMEM in the final concentration of about 57.5 to 62.5 % (v/v), F12 in a final concentration of about 7.5 to 12.5 % (v/v), M171 in a final concentration of about 17.5 to 25.0 % (v/v) and FBS in a final concentration of about 1.75 to 3.5 % (v/v).

48. The cell culture medium of claim 47, wherein the culture medium comprises DMEM in a final concentration of about 61.8 % (v/v), F12 in a final concentration of about 11.8 % (v/v), M171 in a final concentration of about 23.6 % (v/v) and FBS in a final concentration of about 2.5 % (v/v).

49. The cell culture medium of any of claims 46 to 48, wherein the culture medium further comprises Epidermal Growth Factor (EGF) in a final concentration of about 1 ng/ml to about 20 ng/ml.

50. The cell culture medium of any of claims 46 to 49, wherein the culture medium comprise EGF in a final concentration of about 10ng/ml.

51. The cell culture medium of any of claims 46 to 50, wherein the culture medium comprises Insulin in a final concentration of about 1 µg/ml to 10 µg/ml.

52. The cell culture medium of claim 51, wherein the culture medium comprises Insulin in a final concentration of about 5µg/ml.

53. The cell culture medium of any of claims 46 to 52, wherein the culture medium further comprises at least one of the following supplements: adenine, hydrocortisone, and 3,3′,5-Triiodo-L-thyronine sodium salt (T3).

54. The cell culture medium of claim 53, wherein the culture medium comprises all three of adenine, hydrocortisone, and 3,3′,5-Triiodo-L-thyronine sodium salt (T3).

55. The cell culture medium of claim 53 or 54, wherein the culture medium comprises adenine in a final concentration of about 0.05 to about 0.1 µg/ml adenine, hydrocortisone in a final concentration of about 1 to about 10 µg/ml hydrocortisone and/or 3,3′,5-Triiodo-L-thyronine sodium salt (T3) in a final concentration of about 0.5 to about 5 ng/ml.

56. The cell culture medium of any of claims 46 to 55, wherein 500 ml of the cell culture medium comprise:

i. 250 ml of DMEM

ii. 118 ml M171

iii. 118 ml DMEM/F12

iv. 12.5 ml Fetal Bovine Serum (FBS) (final concentration of 2.5%)

57. The cell culture medium of claim 56, further comprising

v. EGF in a final concentration of 10ng/ml

vi. Insulin in a final concentration of 5µg/ml.

vi. Insulin 0.175 ml (final concentration of 5µg/ml)

58. The cell culture medium of claim 56 or 57, further comprising adenine in a final concentration of about 0.05 to about 0.1 µg/ml adenine, hydrocortisone in a final concentration of about 1 to about 10 µg/ml hydrocortisone and/or 3,3′,5-Triiodo-L-thyronine sodium salt (T3) in a final concentration of about 0.5 to about 5 ng/ml.

59. The use of a cell culture medium as defined in any of claims 46 to 58 for isolation of mesenchymal stem cells from the amniotic membrane of umbilical cord.

60. The use of a cell culture medium as defined in any of claims 46 to 58 for cultivation of mesenchymal stem cells from the amniotic membrane of umbilical cord.

Fig. 1

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Document # TS-12-604-3 08/11

Dulbecco's modified eagle medium (DMEM)

Product use

Dulbecco's modified eagle medium was developed in 1969 and is a modification of basal medium eagle (BME) that differs from BME and MEM by the following characteristics:

- Vitamins 4X greater than MEM. Vitamins and amino acids greater than BME
- Types and quantities of amino acids greater than MEM and BME
- Iron (ferric nitrate)

Description

12-604 With 4.5 g/L glucose, with L-glutamine
12-614 With 4.5 g/L glucose, without L-glutamine
12-707 With 1.0 g/L glucose, without L-glutamine
12-708 With 1.0 g/L glucose, and 25 mM HEPES buffer, without L-glutamine
12-709 With 4.5 g/L glucose, and 25 mM HEPES buffer, without L-glutamine
12-733 With 4.5 g/L glucose, without L-glutamine, without sodium pyruvate
12-741 With 4.5 g/L glucose, with L-glutamine, without sodium pyruvate
12-914 With 4.5 g/L glucose, without L-glutamine, screened to support hybridoma growth
12-917 With 4.5 g/L glucose, without L-glutamine or phenol red
15-604 With 4.5 g/L glucose, with L-glutamine, without sodium pyruvate, powder
15-614 With 4.5 g/L glucose, without L-glutamine or sodium pyruvate, powder
(Powder formulations require the addition of 49.3 ml/L of $NaHCO_3$ 7.5% solution or 3.70 g/L of $NaHCO_3$ powder)

Quick reference chart

	Glucose	L-glutamine	Phenol red	HEPES buffer	Sodium pyruvate
12-604	4.5 g/L	+	+	-	+
12-614	4.5 g/L	-	+	-	+
12-707	1.0 g/L	-	+	-	+
12-708	1.0 g/L	-	+	+	+
12-709	4.5 g/L	-	+	+	+
12-733	4.5 g/L	-	+	-	-
12-741	4.5 g/L	+	+	-	-
12-914	4.5 g/L	-	+	-	+
12-917	4.5 g/L	-	-	-	+

Fig. 1 continued

Lonza

Specifications

	Sterility	pH	Osmolality (mOsm)	Cell growth promotion (% of control)	Endotoxin (EU/ml)
12-604	Neg.	7.0-7.4	324-352	≥75%	FIO
12-614	Neg.	7.0-7.4	324-352	≥75%	FIO
12-707	Neg.	7.0-7.4	306-346	≥75%	FIO
12-708	Neg.	7.03-7.27	300-326	≥75%	FIO
12-709	Neg.	7.0-7.4	321-351	≥75%	FIO
12-733	Neg.	7.0-7.4	318-360	≥75%	FIO
12-741	Neg.	7.0-7.4	318-360	≥75%	FIO
12-914	Neg.	7.0-7.4	324-352	≥85% **	FIO
12-917	Neg.	7.0-7.4	329-343	≥75%	FIO
15-604 ***	-	5.5-7.0	235-265	≥75%	≤1.0
15-614 ***	-	5.5-7.0	235-265	≥75%	≤1.0

FIO – for information only
** Result of functional test
*** Moisture content #1.0

Storage

2℃ to 8℃

Product use statement

THESE PRODUCTS ARE FOR RESEARCH USE ONLY. Not approved for human or veterinary use, for application to humans or animals, or for use in clinical or *in vitro* procedures.

Ordering information

Catalog number	Description	Size
12-604F	DMEM with 4.5 g/L glucose, with L-glutamine	500 ml
12-604Q	DMEM with 4.5 g/L glucose, with L-glutamine	1 L
12-614F	DMEM with 4.5 g/L glucose, without L-glutamine	500 ml
12-614Q	DMEM with 4.5 g/L glucose, without L-glutamine	1 L
12-707F	DMEM with 1.0 g/L glucose, without L-glutamine	500 ml
12-708F	DMEM with 1.0 g/L glucose, and 25 mM HEPES buffer, without L-glutamine	500 ml
12-709F	DMEM with 4.5 g/L glucose, and 25 mM HEPES buffer, without L-glutamine	500 ml
12-733F	DMEM with 4.5 g/L glucose, without L-glutamine, without sodium pyruvate	500 ml
12-733Q	DMEM with 4.5 g/L glucose, without L-glutamine, without sodium pyruvate	1 L
12-741F	DMEM with 4.5 g/L glucose, with L-glutamine, without sodium pyruvate	500 ml
12-914F	DMEM with 4.5 g/L glucose, without L-glutamine, screened to support hybridoma growth.	500 ml
12-917F	DMEM with 4.5 g/L glucose, without L-glutamine or phenol red.	500 ml
15-604D	DMEM with 4.5 g/L glucose, with L-glutamine, without sodium pyruvate, powder	1 x 10L
15-604F	DMEM with 4.5 g/L glucose, without L-glutamine or sodium pyruvate, powder	1 X 50L
15-614D	DMEM with 4.5 g/L glucose, without L-glutamine or sodium pyruvate, powder	1 x 10L

Fig. 2

Lonza Walkersville, Inc.
www.lonza.com
biotechserv@lonza.com
Tech Service: 800-521-0390
Document # TS 12-615-2 11/08
Walkersville, MD 21793-0127 USA

Ham's F12 Medium

Product Use

Ham's F12 Medium is a nutrient mixture designed to cultivate a wide variety of mammalian and hybridoma cells when used with serum in combination with hormones and transferrin.

Specifications

Sterility	pH	Osmolality (mOsm)
Negative	7.07-7.40	286-305

Cell Growth Generation	Endotoxin (EU/ml)
≥75% of control	FIO

Storage

2ºC to 8ºC

Product Use Statement

THESE PRODUCTS ARE FOR RESEARCH USE ONLY. Not approved for human or veterinary use, for application to humans or animals, or for use in clinical or *in vitro* procedures.

Ordering Information

Catalog Number	Description	Size
12-615F	Ham's F12 Medium with L-glutamine	500 ml

Lonza

www.lonza.com
U.S. Scientific Support: 800-521-0390
scientific.support@lonza.com
EU/ROW Scientific Support: +49-221-99199-400
scientific.support.eu@lonza.com
Document # TS-12-719-3 08/11

DMEM: F12 (1:1) medium

Product use

DMEM combined with Hams F12 has been extensively used to demonstrate the effect of various hormones and growth factors on target tissues.

Description

12-719	with L-glutamine, 15 mM HEPES, and 3.151 g/L glucose
15-719	powder – with L-glutamine, 15 mM HEPES, and 3.151 g/L glucose. Powdered medium requires the addition of 16.0 ml/L of $NaHCO_3$, 7.5% solution or 1.2 g/L $NaHCO_3$ powder
04-687	with 3.151 g/L glucose, with L-glutamine, without HEPES

Specifications

	Sterility	pH	Osmolality (mOsm)
12-719	Negative	7.0-7.4	286-356
15-719*	Negative	4.5-6.5	260-290
04-687	Negative	FIO	FIO

	Cell growth promotion	Endotoxin (EU/ml)	Moisture
12-719	≥ 75% of control	FIO	--
15-719*	≥ 75% of control	≤ 1 EU/ml	≤ 2%
04-687	--	FIO	--

* pH, osmolality, endotoxin, and moisture are done without $NaHCO_3$; $NaHCO_3$ is added for cell growth test.

Storage

2℃ to 8℃

Product use statement

THESE PRODUCTS ARE FOR RESEARCH USE ONLY. Not approved for human or veterinary use, for application to humans or animals, or for use in clinical or *in vitro* procedures.

Ordering information

Catalog number	Description	Size
12-719F	DMEM: F12 with L-glutamine, 15 mM HEPES, and 3.151 g/L glucose	500 ml
12-719Q	DMEM: F12 with L-glutamine, 15 mM HEPES, and 3.151 g/L glucose	1 L
15-719D	DMEM: F12 powder – with L-glutamine, 15 mM HEPES, and 3.151 g/L glucose. Powdered medium requires the addition of 16.0 ml/L of $NaHCO_3$, 7.5% solution or 1.2 g/L $NaHCO_3$ powder	1 x 10L
04-687Q	DMEM: F12 with 3.151 g/L glucose, with L-glutamine, without HEPES	1 L

Fig. 4

Cascade Biologics™
invitrogen cell culture

GIBCO®
invitrogen cell culture

Medium 171, Medium 171PRF, and MEGS

Medium 171

M-171-500

500 ml

Medium 171PRF (Phenol Red-Free)

M-171PRF-500

500 ml

Product Description

Medium 171 and Medium 171PRF are sterile, liquid tissue culture media intended for use as one component in a complete culture environment for the growth of normal human mammary epithelial cells. Medium 171 is a basal medium containing essential and non-essential amino acids, vitamins, other organic compounds, trace minerals, and inorganic salts. Medium 171PRF is a phenol red-free version of Medium 171. These media do not contain antibiotics, antimycotics, hormones, growth factors, or proteins. These media are HEPES and bicarbonate buffered and are designed for use in an incubator with an atmosphere of 5% CO_2/95% air. To support plating and long-term proliferation of normal human mammary epithelial cells, these media must be supplemented with Mammary Epithelial Growth Supplement (MEGS, cat. no S-015-5).

Intended Use

Medium 171 is intended for use in the routine culture of normal human mammary epithelial cells. Medium 171PRF is intended for use by investigators who wish to culture normal human mammary epithelial cells in the absence of phenol red. When supplemented with MEGS, these media will support the plating and proliferation of normal human mammary epithelial cells at densities between 2.5 x 10^3 cells/cm^2 and 8 x 10^4 cells/cm^2. ***This product is for research use only. Not for use in animals, humans, or diagnostic procedures.***

Caution: If handled improperly, some components of this product may present a health hazard. Take appropriate precautions when handling this product, including the wearing of protective clothing and eyewear. Dispose of properly.

Storage and Stability

Medium 171 and Medium 171PRF are stored at 4° C in our facility and are shipped at ambient temperature. Upon receipt, these media should be stored at 4° C and should not be frozen. **Protect from light.** Several components of these tissue culture media are light-labile, and we recommend that the media not be exposed to light for lengthy periods of time. If the media are warmed prior to use, do not exceed 37° C. When stored in the dark at 4° C, the product is stable until the expiration date on the label.

Preparation of Supplemented Medium 171

1. Thaw one bottle of MEGS. Take one bottle of Medium from cold storage. Make sure that the caps of the vessels are tight.
2. Gently swirl the bottle of supplement. Avoid splashing the supplement into the cap of the bottle or causing the supplement to foam.
3. Wipe the outside of the containers with a disinfecting solution such as 70% ethanol or isopropanol.
4. Using sterile technique in a laminar flow culture hood, transfer the entire contents of the bottle of supplement to the bottle of Medium.
5. Tightly cap the bottle of supplemented medium and swirl the contents to ensure a homogeneous solution. Avoid causing the medium to foam.

Storage and Stability of Supplemented Medium 171

Once Medium 171 or Medium 171PRF has been supplemented with MEGS, the supplemented medium should be stored in the dark at 4° C and should not be frozen. When stored in the dark at 4° C, the supplemented medium is stable for 1 month.

Selected References

The Medium 171 formulation is based on medium MCDB 170, with modifications.

Hammond SL, Ham RG, Stampfer MR; PNAS 81:5435-5439, 1984

Fig. 4 continued

6/12

Cascade Biologics™
invitrogen cell culture

GIBCO®
invitrogen cell culture

MEGS
Mammary Epithelial Growth Supplement

Cat. no. S-015-5
5 ml

Product Description

Mammary Epithelial Growth Supplement (MEGS) is a sterile, concentrated (100X) solution intended for use as one component in a complete culture environment for the growth of normal human mammary epithelial cells. Each 5 ml bottle of MEGS contains all of the growth factors, hormones, and tissue extracts necessary for the culture of normal human mammary epithelial cells and is the correct amount of supplement for a 500 ml bottle of Medium 171 or Medium 171PRF. MEGS is an ionically-balanced supplement containing bovine pituitary extract (BPE), bovine insulin, hydrocortisone and recombinant human epidermal growth factor. When a 500 ml bottle of Medium 171 or Medium 171PRF is supplemented with MEGS, the final concentrations of the components in the supplemented medium are: BPE, 0.4% v/v; bovine insulin, 5 µg/ml; hydrocortisone, 0.5 µg/ml; and recombinant human epidermal growth factor, 3 ng/ml.

Intended Use

MEGS is intended for use in conjunction with Medium 171 or Medium 171PRF for the routine serum-free culture of normal human mammary epithelial cells. ***This product is for research use only. Not for use in animals, humans, or diagnostic procedures.***

Caution: If handled improperly, some components of this product may present a health hazard. Take appropriate precautions when handling this product, including the wearing of protective clothing and eyewear. Dispose of properly.

Storage and Stability

MEGS is stored at –20° C at our facility and is shipped on dry ice. Upon receipt, the product should be stored at –20° C in a freezer that is not self-defrosting. When stored at –20° C, the product is stable until the expiration date shown on the label.

After long-term storage at –20° C, MEGS may contain a small amount of precipitate. This precipitate is formed from cold-insoluble material in the BPE component of the MEGS and will not affect the performance of the product.

Thawing

To thaw, place the product in a 37° C water bath or overnight at 4° C. If thawed in a water bath, do not leave the product at 37° C after the product has thawed. For instructions on adding MEGS to Medium 171, please refer to the instructions that accompany the basal medium.

Selected References

The MEGS formulation is based on published supplementation of medium MCDB 170, with modifications.

Hammond SL, Ham RG, Stampfer MR; PNAS 81:5435-5439, 1984.

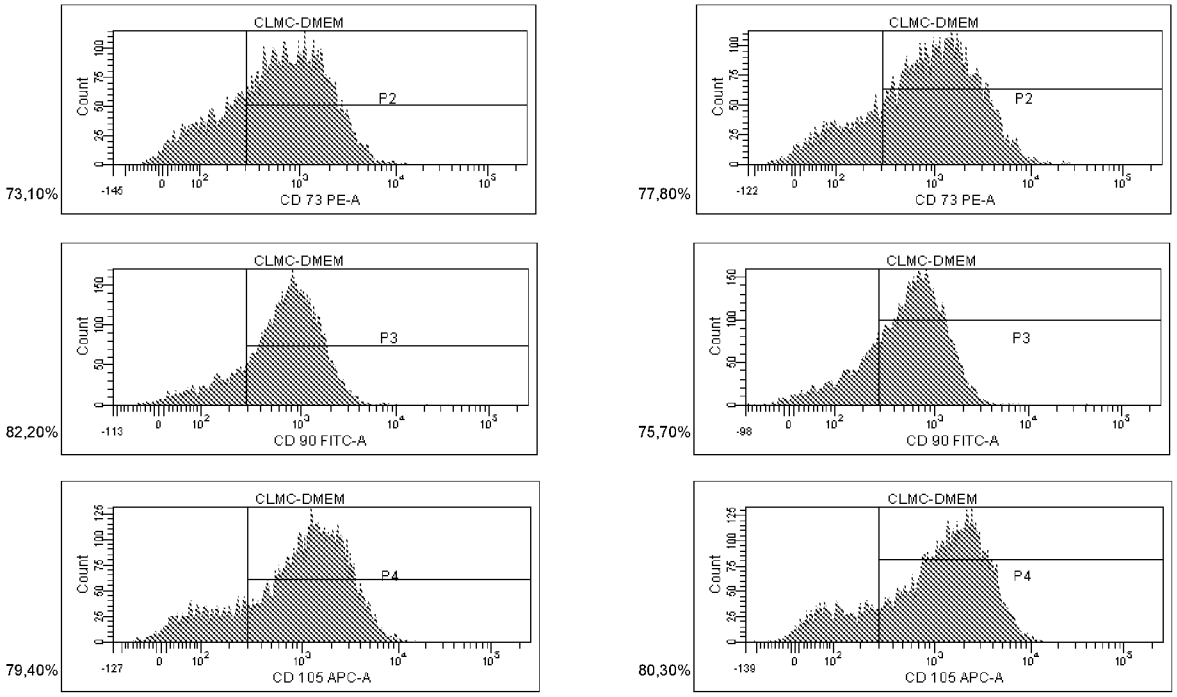
For research use only.

Life Technologies Corporation • 5791 Van Allen Way • Carlsbad • CA 92008 • Tel: 800.955.6288 • www.invitrogen.com • E-mail: tech_support@invitrogen.com

Fig. 5

PTT6 Medium ingredients list

Medium composition List	Company Name	Catalogue Number
Basic media		
DMEM (also referred to herein as PTT6-basal medium)	Lonza	12-604F
DMEM/F12	Lonza	12-719F
M171	Life technologies	M171500
Serum		
Fetal Bovine Serum	GE Healthcare	A15-151
Antibiotic		
Penicillin-Streptomycin-Amphotericin B	Lonza	17-745E
Supplements		
Adenine (optional)	Sigma	A8626-25G
Hydrocortisone (optional)	Sigma	H-0888
Epidermal Growth Factor	Millipore	GF-144
T3 (3,3′,5-Triiodo-L-thyronine sodium salt) (optional)	Sigma	200-223-5
Recombinant Human Insulin AOF	Life Technologies	A11382IJ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Count
P2
CD 73 PE-A
73,10%
CLMC-DMEM
Count
P2
CD 73 PE-A
77,80%
CLMC-DMEM
Count
P3
CD 90 FITC-A
82,20%
CLMC-DMEM
Count
P3
CD 90 FITC-A
75,70%
CLMC-DMEM
Count
P4
CD 105 APC-A
79,40%
CLMC-DMEM
Count
P4
CD 105 APC-A
80,30%

Fig. 6a

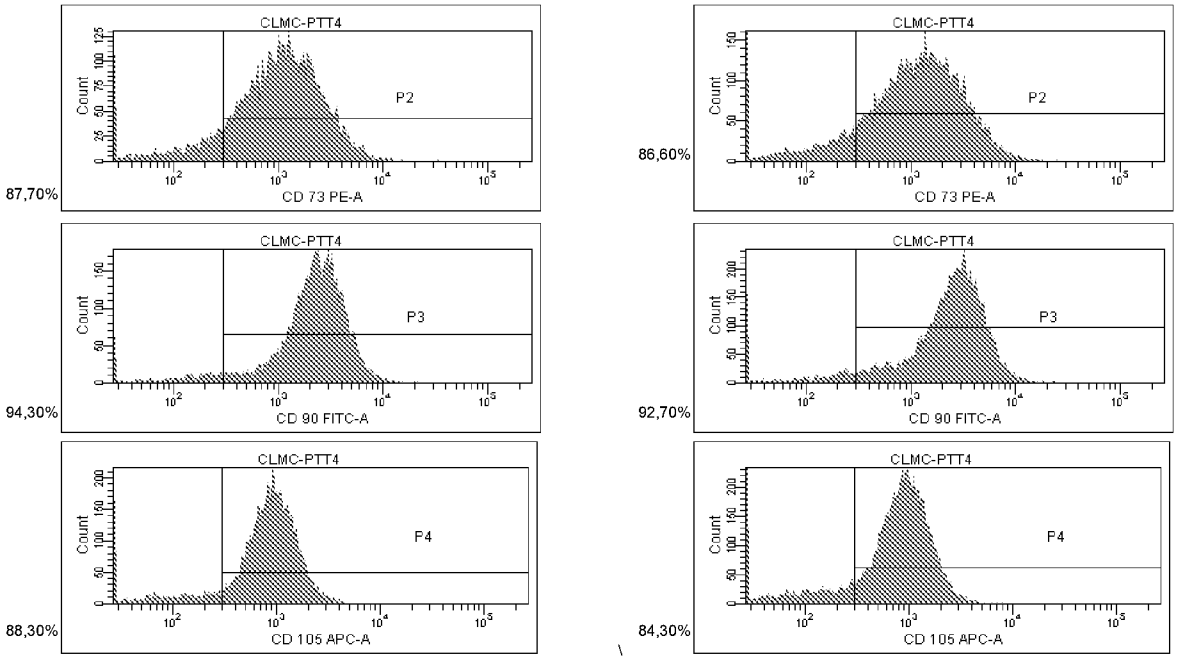

CLMC-PTT4
Count
P2
CD 73 PE-A
87,70%
86,60%
CLMC-PTT4
Count
P3
CD 90 FITC-A
94,30%
92,70%
CLMC-PTT4
Count
P4
CD 105 APC-A
88,30%
84,30%

Fig. 6b

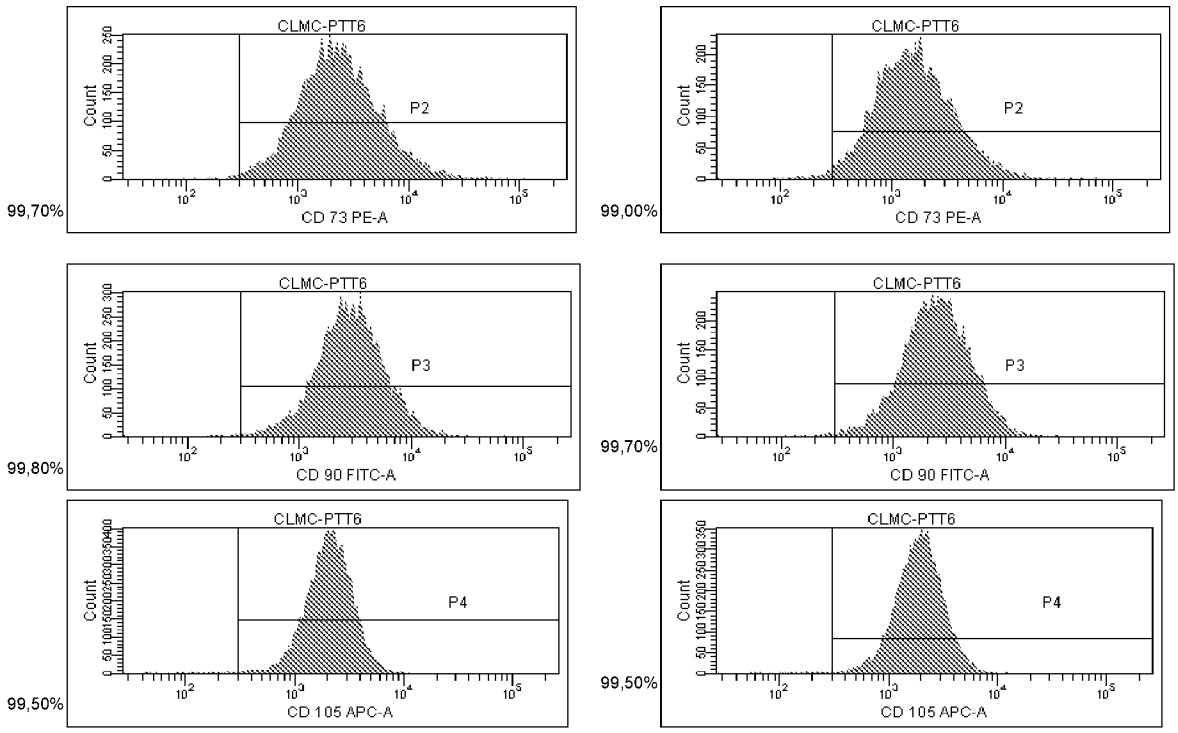

CLMC-PTT6
Count
P2
CD 73 PE-A
99,70%
CLMC-PTT6
Count
P2
CD 73 PE-A
99,00%
CLMC-PTT6
Count
P3
CD 90 FITC-A
99,80%
CLMC-PTT6
Count
P3
CD 90 FITC-A
99,70%
CLMC-PTT6
Count
P4
CD 105 APC-A
99,50%
CLMC-PTT6
Count
P4
CD 105 APC-A
99,50%

Fig. 6c

Fig. 7a

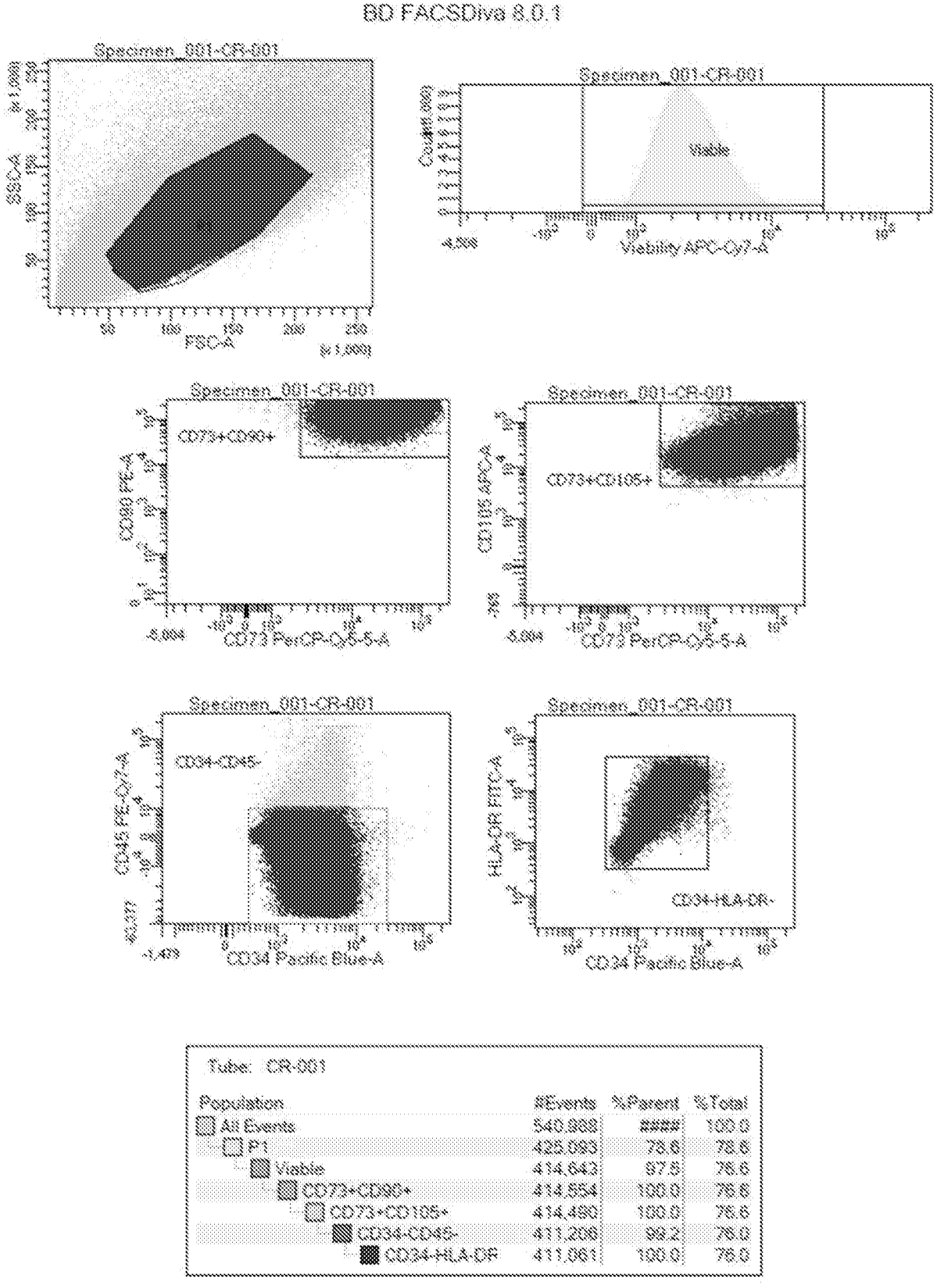
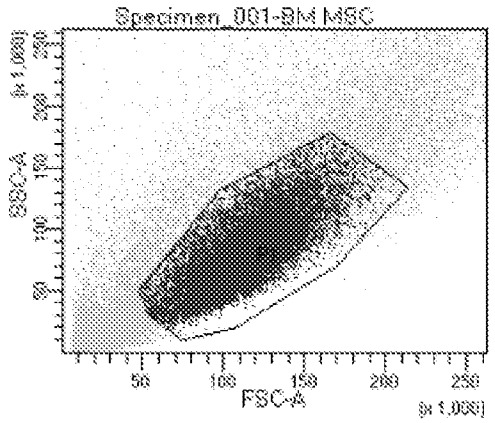
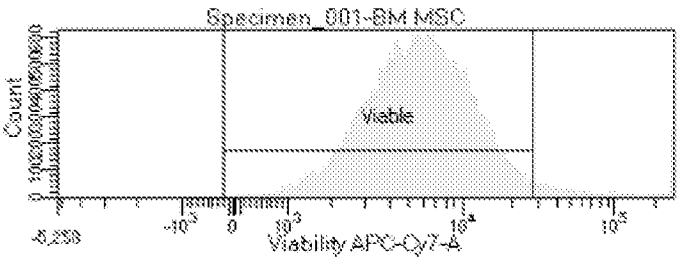
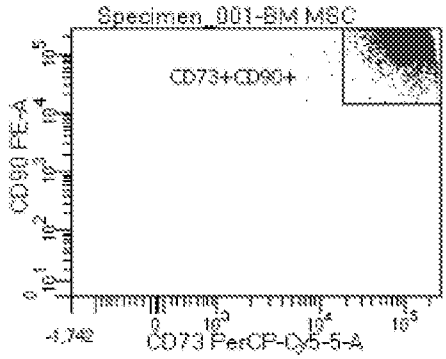
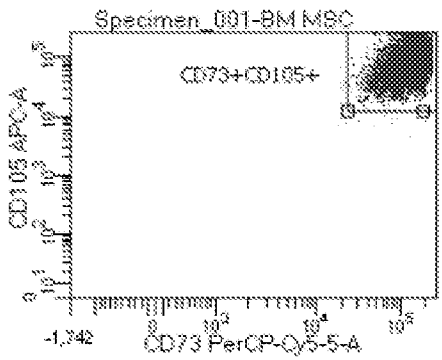

BD FACSDiva 8.0.1
Specimen_001-CR-001
SSC-A
FSC-A
Viable
Viability APC-Cy7-A
CD73+CD90+
CD90 PE-A
CD73 PerCP-Cy5-5-A
CD73+CD105+
CD105 APC-A
CD34-CD45-
CD45 PE-Cy7-A
CD34 Pacific Blue-A
CD34-HLA-DR-
HLA-DR FITC-A
Tube: CR-001
Population #Events %Parent %Total
All Events 540,000 #### 100.0
P1 425,093 78.8 78.8
Viable 414,642 97.5 76.8
CD73+CD90+ 414,554 100.0 76.8
CD73+CD105+ 414,450 100.0 76.8
CD34-CD45- 411,206 99.2 76.0
CD34-HLA-DR- 411,061 100.0 76.0

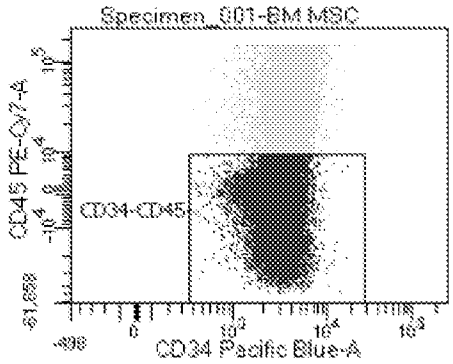
Fig. 7b

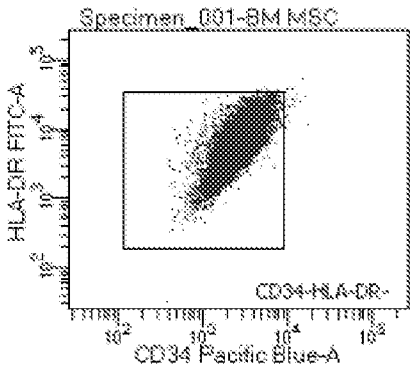












Experiment Name: MSC 062216
Specimen Name: Specimen_001
Tube Name: BM MSC

Population	#Events	%Parent
CD34-HLA-DR-	21,128	99.9

Population	#Events	%Parent	%Total
All Events	77,022	####	100.0
P1	35,780	46.5	46.5
Viable	33,741	94.3	43.8
CD73+CD90+	33,729	100.0	43.8
CD73+CD105+	33,710	99.9	43.8
CD34-CD45-	21,155	62.8	27.5
CD34-HLA-DR-	21,128	99.9	27.4

Fig. 1

International Application Number: SG2017050500
Article 34 Amendments
submitted with Demand for IPEA dated 26 Mar 2018

Lonza

www.lonza.com
U.S. Scientific Support: 800-521-0390
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EU/ROW Scientific Support: +49-221-99199-400
scientific.support.eu@lonza.com
Document # TS-12-604-3 08/11

Dulbecco's modified eagle medium (DMEM)

Product use

Dulbecco's modified eagle medium was developed in 1969 and is a modification of basal medium eagle (BME) that differs from BME and MEM by the following characteristics:

- Vitamins 4X greater than MEM. Vitamins and amino acids greater than BME
- Types and quantities of amino acids greater than MEM and BME
- Iron (ferric nitrate)

Description

12-604 With 4.5 g/L glucose, with L-glutamine
12-614 With 4.5 g/L glucose, without L-glutamine
12-707 With 1.0 g/L glucose, without L-glutamine
12-708 With 1.0 g/L glucose, and 25 mM HEPES buffer, without L-glutamine
12-709 With 4.5 g/L glucose, and 25 mM HEPES buffer, without L-glutamine
12-733 With 4.5 g/L glucose, without L-glutamine, without sodium pyruvate
12-741 With 4.5 g/L glucose, with L-glutamine, without sodium pyruvate
12-914 With 4.5 g/L glucose, without L-glutamine, screened to support hybridoma growth
12-917 With 4.5 g/L glucose, without L-glutamine or phenol red
15-604 With 4.5 g/L glucose, with L-glutamine, without sodium pyruvate, powder
15-614 With 4.5 g/L glucose, without L-glutamine or sodium pyruvate, powder
(Powder formulations require the addition of 49.3 ml/L of $NaHCO_3$ 7.5% solution or 3.70 g/L of $NaHCO_3$ powder)

Quick reference chart

	Glucose	L-glutamine	Phenol red	HEPES buffer	Sodium pyruvate
12-604	4.5 g/L	+	+	-	+
12-614	4.5 g/L	-	+	-	+
12-707	1.0 g/L	-	+	-	+
12-708	1.0 g/L	-	+	+	+
12-709	4.5 g/L	-	+	+	+
12-733	4.5 g/L	-	+	-	-
12-741	4.5 g/L	+	+	-	-
12-914	4.5 g/L	-	+	-	+
12-917	4.5 g/L	-	-	-	+

Fig. 1 continued

Lonza

Specifications

	Sterility	pH	Osmolality (mOsm)	Cell growth promotion (% of control)	Endotoxin (EU/ml)
12-604	Neg.	7.0-7.4	324-352	≥75%	FIO
12-614	Neg.	7.0-7.4	324-352	≥75%	FIO
12-707	Neg.	7.0-7.4	306-346	≥75%	FIO
12-708	Neg.	7.03-7.27	300-326	≥75%	FIO
12-709	Neg.	7.0-7.4	321-351	≥75%	FIO
12-733	Neg.	7.0-7.4	318-360	≥75%	FIO
12-741	Neg.	7.0-7.4	318-360	≥75%	FIO
12-914	Neg.	7.0-7.4	324-352	≥85% **	FIO
12-917	Neg.	7.0-7.4	329-343	≥75%	FIO
15-604 ***	-	5.5-7.0	235-265	≥75%	≤1.0
15-614 ***	-	5.5-7.0	235-265	≥75%	≤1.0

FIO – for information only
** Result of functional test
*** Moisture content #1.0

Storage

2℃ to 8℃

Product use statement

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Ordering information

Catalog number	Description	Size
12-604F	DMEM with 4.5 g/L glucose, with L-glutamine	500 ml
12-604Q	DMEM with 4.5 g/L glucose, with L-glutamine	1 L
12-614F	DMEM with 4.5 g/L glucose, without L-glutamine	500 ml
12-614Q	DMEM with 4.5 g/L glucose, without L-glutamine	1 L
12-707F	DMEM with 1.0 g/L glucose, without L-glutamine	500 ml
12-708F	DMEM with 1.0 g/L glucose, and 25 mM HEPES buffer, without L-glutamine	500 ml
12-709F	DMEM with 4.5 g/L glucose, and 25 mM HEPES buffer, without L-glutamine	500 ml
12-733F	DMEM with 4.5 g/L glucose, without L-glutamine, without sodium pyruvate	500 ml
12-733Q	DMEM with 4.5 g/L glucose, without L-glutamine, without sodium pyruvate	1 L
12-741F	DMEM with 4.5 g/L glucose, with L-glutamine, without sodium pyruvate	500 ml
12-914F	DMEM with 4.5 g/L glucose, without L-glutamine, screened to support hybridoma growth.	500 ml
12-917F	DMEM with 4.5 g/L glucose, without L-glutamine or phenol red.	500 ml
15-604D	DMEM with 4.5 g/L glucose, with L-glutamine, without sodium pyruvate, powder	1 x 10L
15-604F	DMEM with 4.5 g/L glucose, without L-glutamine or sodium pyruvate, powder	1 X 50L
15-614D	DMEM with 4.5 g/L glucose, without L-glutamine or sodium pyruvate, powder	1 x 10L

Fig. 2

Lonza Walkersville, Inc.
www.lonza.com
biotechserv@lonza.com
Tech Service: 800-521-0390
Document # TS 12-615-2 11/08
Walkersville, MD 21793-0127 USA

Ham's F12 Medium

Product Use

Ham's F12 Medium is a nutrient mixture designed to cultivate a wide variety of mammalian and hybridoma cells when used with serum in combination with hormones and transferrin.

Specifications

Sterility	pH	Osmolality (mOsm)
Negative	7.07-7.40	286-305

Cell Growth Generation	Endotoxin (EU/ml)
≥75% of control	FIO

Storage

2ºC to 8ºC

Product Use Statement

THESE PRODUCTS ARE FOR RESEARCH USE ONLY. Not approved for human or veterinary use, for application to humans or animals, or for use in clinical or *in vitro* procedures.

Ordering Information

Catalog Number	Description	Size
12-615F	Ham's F12 Medium with L-glutamine	500 ml

Lonza

www.lonza.com
U.S. Scientific Support: 800-521-0390
scientific.support@lonza.com
EU/ROW Scientific Support: +49-221-99199-400
scientific.support.eu@lonza.com
Document # TS-12-719-3 08/11

DMEM: F12 (1:1) medium

Product use

DMEM combined with Hams F12 has been extensively used to demonstrate the effect of various hormones and growth factors on target tissues.

Description

12-719	with L-glutamine, 15 mM HEPES, and 3.151 g/L glucose
15-719	powder – with L-glutamine, 15 mM HEPES, and 3.151 g/L glucose. Powdered medium requires the addition of 16.0 ml/L of $NaHCO_3$, 7.5% solution or 1.2 g/L $NaHCO_3$ powder
04-687	with 3.151 g/L glucose, with L-glutamine, without HEPES

Specifications

	Sterility	pH	Osmolality (mOsm)
12-719	Negative	7.0-7.4	286-356
15-719*	Negative	4.5-6.5	260-290
04-687	Negative	FIO	FIO

	Cell growth promotion	Endotoxin (EU/ml)	Moisture
12-719	≥ 75% of control	FIO	--
15-719*	≥ 75% of control	≤ 1 EU/ml	≤ 2%
04-687	--	FIO	--

* pH, osmolality, endotoxin, and moisture are done without $NaHCO_3$; $NaHCO_3$ is added for cell growth test.

Storage

2℃ to 8℃

Product use statement

THESE PRODUCTS ARE FOR RESEARCH USE ONLY. Not approved for human or veterinary use, for application to humans or animals, or for use in clinical or *in vitro* procedures.

Ordering information

Catalog number	Description	Size
12-719F	DMEM: F12 with L-glutamine, 15 mM HEPES, and 3.151 g/L glucose	500 ml
12-719Q	DMEM: F12 with L-glutamine, 15 mM HEPES, and 3.151 g/L glucose	1 L
15-719D	DMEM: F12 powder – with L-glutamine, 15 mM HEPES, and 3.151 g/L glucose. Powdered medium requires the addition of 16.0 ml/L of $NaHCO_3$, 7.5% solution or 1.2 g/L $NaHCO_3$ powder	1 x 10L
04-687Q	DMEM: F12 with 3.151 g/L glucose, with L-glutamine, without HEPES	1 L

Fig. 4

Cascade Biologics™
invitrogen cell culture

GIBCO®
invitrogen cell culture

Medium 171, Medium 171PRF, and MEGS

Medium 171

M-171-500

500 ml

Medium 171PRF (Phenol Red-Free)

M-171PRF-500

500 ml

Product Description

Medium 171 and Medium 171PRF are sterile, liquid tissue culture media intended for use as one component in a complete culture environment for the growth of normal human mammary epithelial cells. Medium 171 is a basal medium containing essential and non-essential amino acids, vitamins, other organic compounds, trace minerals, and inorganic salts. Medium 171PRF is a phenol red-free version of Medium 171. These media do not contain antibiotics, antimycotics, hormones, growth factors, or proteins. These media are HEPES and bicarbonate buffered and are designed for use in an incubator with an atmosphere of 5% CO_2/95% air. To support plating and long-term proliferation of normal human mammary epithelial cells, these media must be supplemented with Mammary Epithelial Growth Supplement (MEGS, cat. no S-015-5).

Intended Use

Medium 171 is intended for use in the routine culture of normal human mammary epithelial cells. Medium 171PRF is intended for use by investigators who wish to culture normal human mammary epithelial cells in the absence of phenol red. When supplemented with MEGS, these media will support the plating and proliferation of normal human mammary epithelial cells at densities between 2.5 x 10^3 cells/cm^2 and 8 x 10^4 cells/cm^2. ***This product is for research use only. Not for use in animals, humans, or diagnostic procedures.***

Caution: If handled improperly, some components of this product may present a health hazard. Take appropriate precautions when handling this product, including the wearing of protective clothing and eyewear. Dispose of properly.

Storage and Stability

Medium 171 and Medium 171PRF are stored at 4° C in our facility and are shipped at ambient temperature. Upon receipt, these media should be stored at 4° C and should not be frozen. **Protect from light.** Several components of these tissue culture media are light-labile, and we recommend that the media not be exposed to light for lengthy periods of time. If the media are warmed prior to use, do not exceed 37° C. When stored in the dark at 4° C, the product is stable until the expiration date on the label.

Preparation of Supplemented Medium 171

1. Thaw one bottle of MEGS. Take one bottle of Medium from cold storage. Make sure that the caps of the vessels are tight.
2. Gently swirl the bottle of supplement. Avoid splashing the supplement into the cap of the bottle or causing the supplement to foam.
3. Wipe the outside of the containers with a disinfecting solution such as 70% ethanol or isopropanol.
4. Using sterile technique in a laminar flow culture hood, transfer the entire contents of the bottle of supplement to the bottle of Medium.
5. Tightly cap the bottle of supplemented medium and swirl the contents to ensure a homogeneous solution. Avoid causing the medium to foam.

Storage and Stability of Supplemented Medium 171

Once Medium 171 or Medium 171PRF has been supplemented with MEGS, the supplemented medium should be stored in the dark at 4° C and should not be frozen. When stored in the dark at 4° C, the supplemented medium is stable for 1 month.

Selected References

The Medium 171 formulation is based on medium MCDB 170, with modifications.

Hammond SL, Ham RG, Stampfer MR; PNAS 81:5435-5439, 1984

Fig. 4 continued

6/12

Cascade Biologics™
invitrogen cell culture

GIBCO®
invitrogen cell culture

MEGS
Mammary Epithelial Growth Supplement

Cat. no. S-015-5
5 ml

Product Description

Mammary Epithelial Growth Supplement (MEGS) is a sterile, concentrated (100X) solution intended for use as one component in a complete culture environment for the growth of normal human mammary epithelial cells. Each 5 ml bottle of MEGS contains all of the growth factors, hormones, and tissue extracts necessary for the culture of normal human mammary epithelial cells and is the correct amount of supplement for a 500 ml bottle of Medium 171 or Medium 171PRF. MEGS is an ionically-balanced supplement containing bovine pituitary extract (BPE), bovine insulin, hydrocortisone and recombinant human epidermal growth factor. When a 500 ml bottle of Medium 171 or Medium 171PRF is supplemented with MEGS, the final concentrations of the components in the supplemented medium are: BPE, 0.4% v/v; bovine insulin, 5 µg/ml; hydrocortisone, 0.5 µg/ml; and recombinant human epidermal growth factor, 3 ng/ml.

Intended Use

MEGS is intended for use in conjunction with Medium 171 or Medium 171PRF for the routine serum-free culture of normal human mammary epithelial cells. ***This product is for research use only. Not for use in animals, humans, or diagnostic procedures.***

Caution: If handled improperly, some components of this product may present a health hazard. Take appropriate precautions when handling this product, including the wearing of protective clothing and eyewear. Dispose of properly.

Storage and Stability

MEGS is stored at –20° C at our facility and is shipped on dry ice. Upon receipt, the product should be stored at –20° C in a freezer that is not self-defrosting. When stored at –20° C, the product is stable until the expiration date shown on the label.

After long-term storage at –20° C, MEGS may contain a small amount of precipitate. This precipitate is formed from cold-insoluble material in the BPE component of the MEGS and will not affect the performance of the product.

Thawing

To thaw, place the product in a 37° C water bath or overnight at 4° C. If thawed in a water bath, do not leave the product at 37° C after the product has thawed. For instructions on adding MEGS to Medium 171, please refer to the instructions that accompany the basal medium.

Selected References

The MEGS formulation is based on published supplementation of medium MCDB 170, with modifications.

Hammond SL, Ham RG, Stampfer MR; PNAS 81:5435-5439, 1984.

For research use only.

Life Technologies Corporation • 5791 Van Allen Way • Carlsbad • CA 92008 • Tel: 800.955.6288 • www.invitrogen.com • E-mail: tech_support@invitrogen.com

Fig. 5

PTT6 Medium ingredients list

Medium composition List	Company Name	Catalogue Number
Basic media		
DMEM (also referred to herein as PTT6-basal medium)	Lonza	12-604F
DMEM/F12	Lonza	12-719F
M171	Life technologies	M171500
Serum		
Fetal Bovine Serum	GE Healthcare	A15-151
Antibiotic		
Penicillin-Streptomycin-Amphotericin B	Lonza	17-745E
Supplements		
Adenine (optional)	Sigma	A8626-25G
Hydrocortisone (optional)	Sigma	H-0888
Epidermal Growth Factor	Millipore	GF-144
T3 (3,3′,5-Triiodo-L-thyronine sodium salt) (optional)	Sigma	200-223-5
Recombinant Human Insulin AOF	Life Technologies	A11382IJ

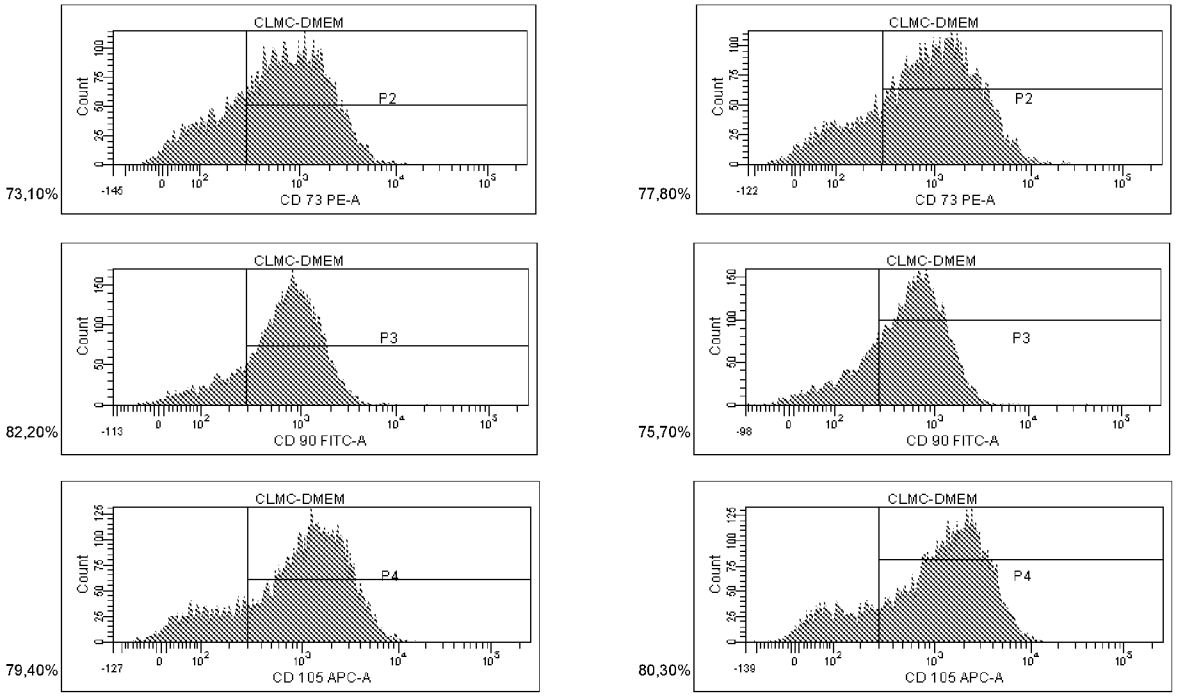

CLMC-DMEM
Count
P2
CD 73 PE-A
73,10%
CLMC-DMEM
Count
P2
CD 73 PE-A
77,80%
CLMC-DMEM
Count
P3
CD 90 FITC-A
82,20%
CLMC-DMEM
Count
P3
CD 90 FITC-A
75,70%
CLMC-DMEM
Count
P4
CD 105 APC-A
79,40%
CLMC-DMEM
Count
P4
CD 105 APC-A
80,30%

Fig. 6a

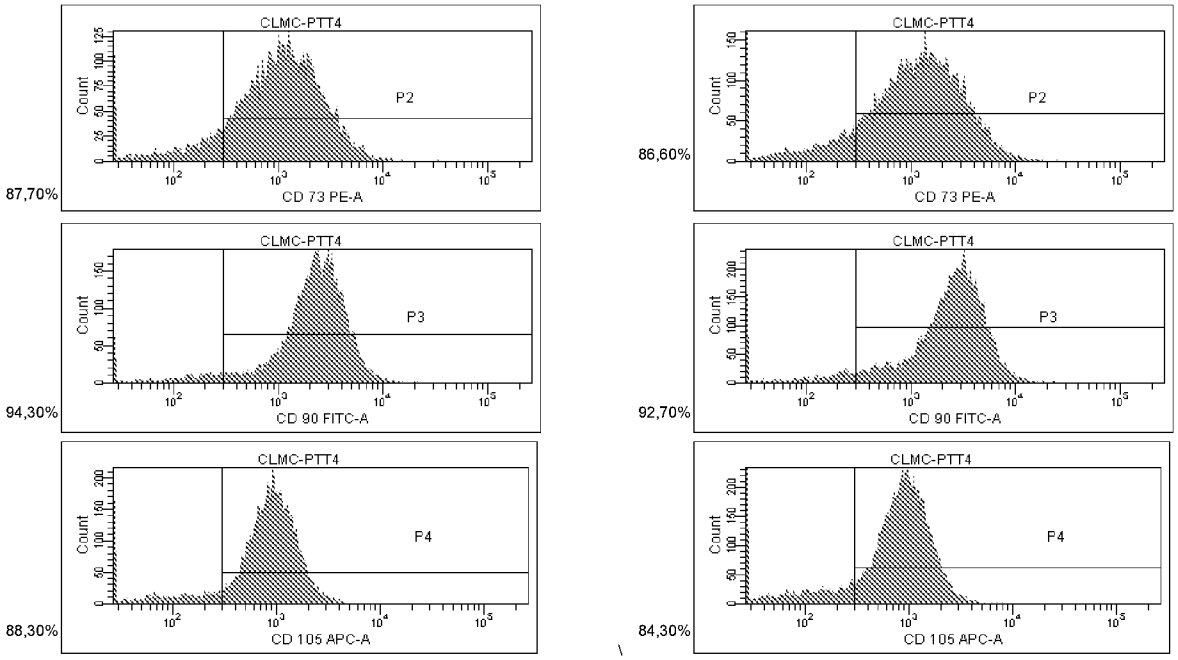

CLMC-PTT4
Count
P2
CD 73 PE-A
87,70%
86,60%
CLMC-PTT4
Count
P3
CD 90 FITC-A
94,30%
92,70%
CLMC-PTT4
Count
P4
CD 105 APC-A
88,30%
84,30%

Fig. 6b

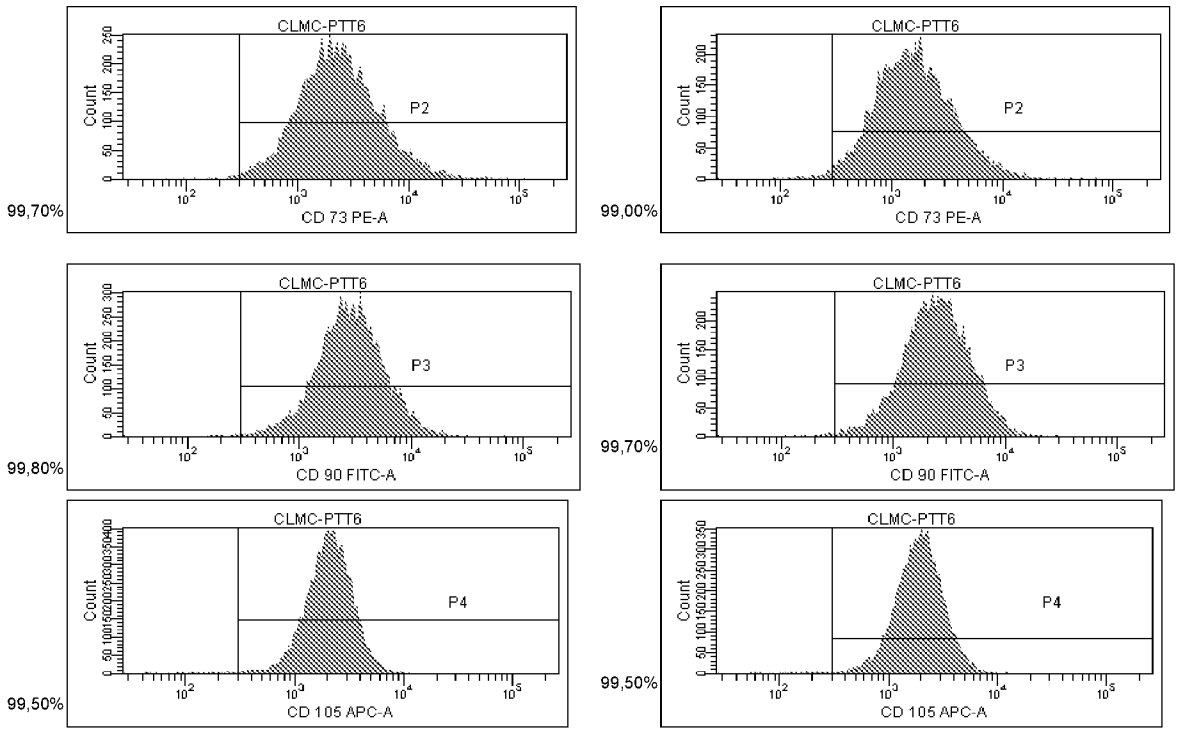

CLMC-PTT6
Count
P2
CD 73 PE-A
99,70%
CLMC-PTT6
Count
P2
CD 73 PE-A
99,00%
CLMC-PTT6
Count
P3
CD 90 FITC-A
99,80%
CLMC-PTT6
Count
P3
CD 90 FITC-A
99,70%
CLMC-PTT6
Count
P4
CD 105 APC-A
99,50%
CLMC-PTT6
Count
P4
CD 105 APC-A
99,50%

Fig. 6c

Fig. 7a

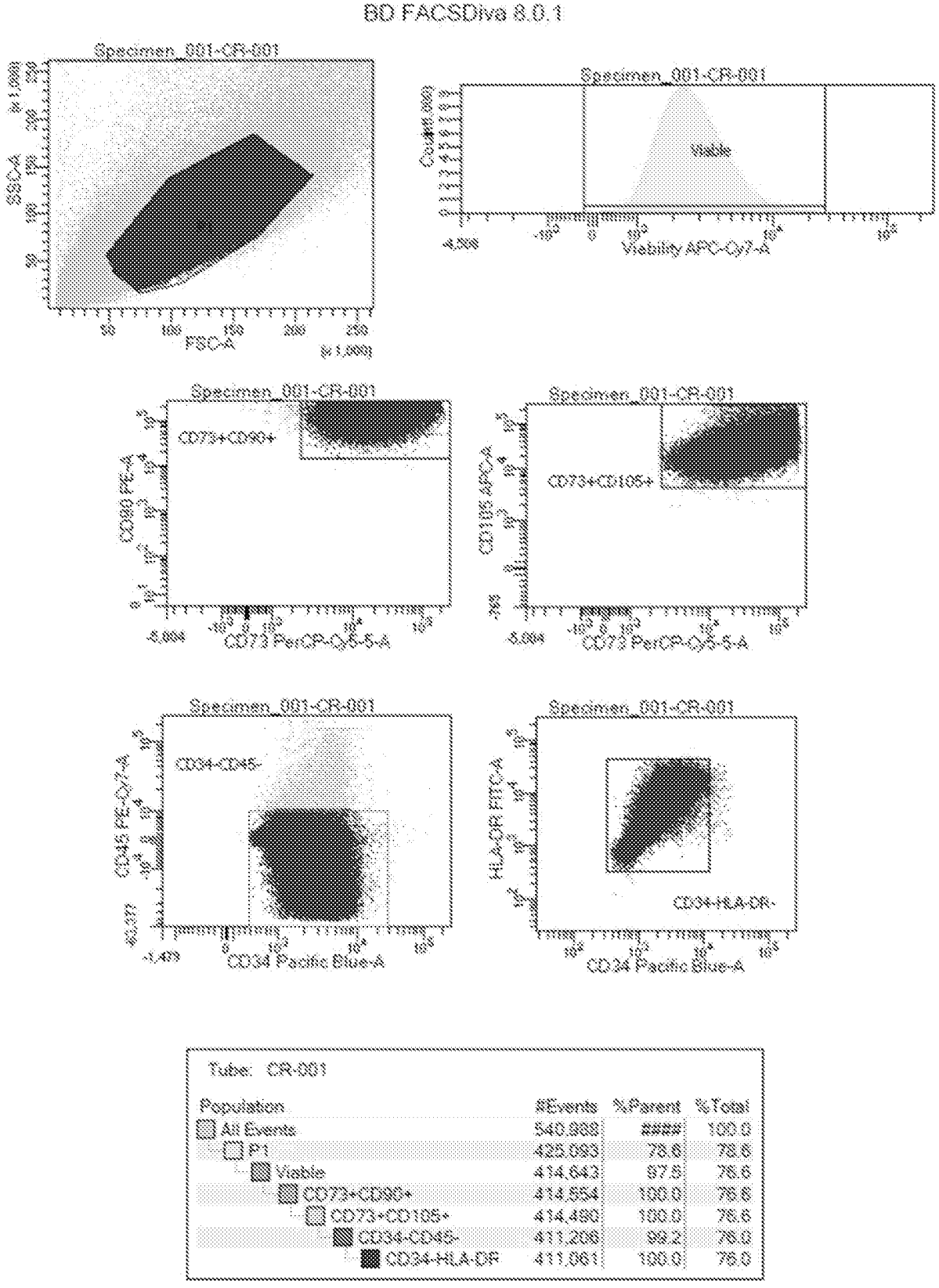
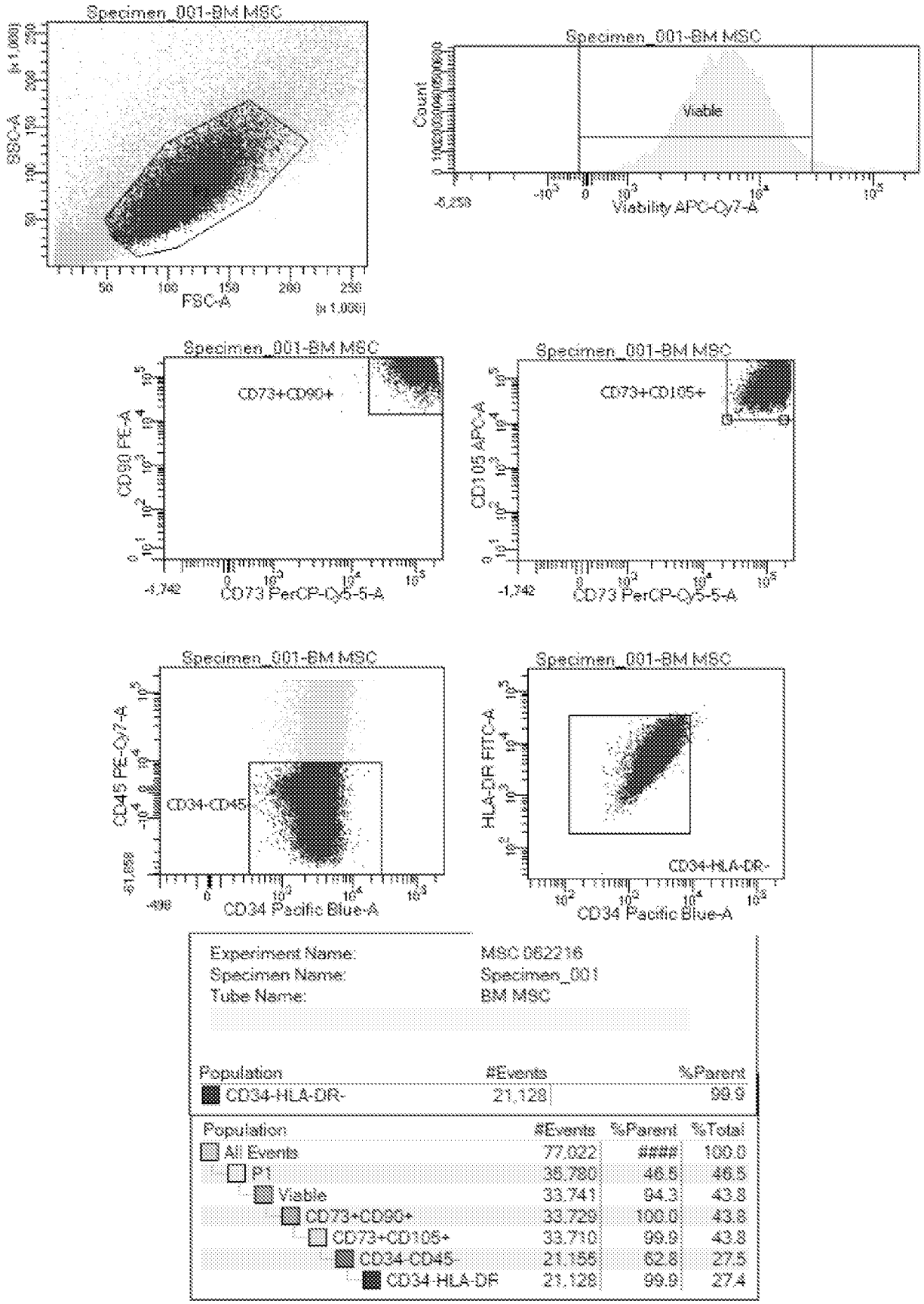

BD FACSDiva 8.0.1
Specimen_001-CR-001
SSC-A
FSC-A
Viable
Viability APC-Cy7-A
CD73+CD90+
CD90 PE-A
CD73 PerCP-Cy5-5-A
CD73+CD105+
CD105 APC-A
CD34-CD45-
CD45 PE-Cy7-A
CD34 Pacific Blue-A
CD34-HLA-DR-
HLA-DR FITC-A
Tube: CR-001
Population #Events %Parent %Total
All Events 540,000 #### 100.0
P1 425,093 78.8 78.8
Viable 414,642 97.5 76.8
CD73+CD90+ 414,554 100.0 76.8
CD73+CD105+ 414,450 100.0 76.8
CD34-CD45- 411,206 99.2 76.0
CD34-HLA-DR- 411,061 100.0 76.0

Fig. 7b



Experiment Name:	MSC 062216
Specimen Name:	Specimen_001
Tube Name:	BM MSC

Population	#Events	%Parent
CD34-HLA-DR-	21,128	99.9

Population	#Events	%Parent	%Total
All Events	77,022	####	100.0
P1	35,780	46.5	46.5
Viable	33,741	94.3	43.8
CD73+CD90+	33,729	100.0	43.8
CD73+CD105+	33,710	99.9	43.8
CD34-CD45-	21,155	62.8	27.5
CD34-HLA-DR-	21,128	99.9	27.4

Fig. 1

Lonza

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U.S. Scientific Support: 800-521-0390
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scientific.support.eu@lonza.com
Document # TS-12-604-3 08/11

Medio Eagle modificado de Dulbecco (DMEM)

Uso del producto

El medio Eagle modificado de Dulbecco fue desarrollado en 1969 y es una modificación del medio Eagle basal (BME) que difiere de BME y MEM por las siguientes características:

- Vitaminas 4 veces mayores que MEM. Vitaminas y aminoácidos mayores que BME
- Tipos y cantidades de aminoácidos mayores que MEM y BME
- Hierro (nitrato de hierro)

Descripción

12-604 Con 4.5 g/L de glucosa, con L-glutamina 12-614 Con 4.5 g/L de glucosa, sin L-glutamina 12-707 Con 1.0 g/L de glucosa, sin L-708 Con 1.0 g/L de glucosa, y 25 mM de agente amortiguador de HEPES, sin L-glutamina

12-709 Con 4,5 g/L de glucosa y 25 mM de agente amortiguador de HEPES, sin L-glutamina

12-733 Con 4.5 g/L de glucosa, sin L-glutamina, sin piruvato de sodio

12-741 Con 4,5 g/L de glucosa, con L-glutamina, sin piruvato sódico

12-914 Con 4.5 g/L de glucosa, sin L-glutamina, examinados para apoyar el crecimiento del hibridoma 12-917 Con 4.5 g/L de glucosa, sin L-glutamina o rojo fenol

15-604 Con 4.5 g/L de glucosa, con L-glutamina, sin piruvato sódico, en polvo

15-614 Con 4.5 g/L de glucosa, sin L-glutamina ni piruvato sódico, polvo (las formulaciones en polvo requieren la adición de 49.3 ml/L de solución de $NaHCO_3$ al 7.5% o 3.70 g/L de $NaHCO_3$ en polvo)

Gráfico de referencia rápida

	Glucosa L-	glutamina	Fenol rojo	búfer HEPES	Sodio piruvato
12-604	4.5 g/L	+	+	-	+
12-614	4.5 g/L	-	+	-	+
12-707	1.0 g/L	-	+	-	+
12-708	1.0 g/L	-	+	+	+
12-709	4.5 g/L	-	+	+	+
12-733	4.5 g/L	-	+	-	-
12-741	4.5 g/L	+	+	-	-
12-914	4.5 g/L	-	+	-	+
12-917	4.5 g/L	-	-	-	+

Fig. 1
continuación

Especificaciones

	Esterilidad	PH	Osmolalidad (mOsm)	Promoción del crecimiento celular (% de control)	Endotoxina (EU/ml)
0.0.0 LU					
12-604	Neg.	7.0-7.4	324-352	>75%	FIO
12-614	Neg.	7.0-7.4	324-352	>75%	FIO
12-707	Neg.	7.0-7.4	306-346	>75%	FIO
12-708	Neg.	7.03 7.27	300-326	>75%	FIO
12-709	Neg.	7.0-7.4	321-351	>75%	FIO
12-733	Neg.	7.0-7.4	318-360	>75%	FIO
12-741	Neg.	7.0-7.4	318-360	>75%	FIO
12-914	Neg.	7.0-7.4	324-352	>85% **	FIO
12-917	Neg.	7.0-7.4	329-343	>75%	FIO
15-604 ***	-	5.5-7.0	235-265	>75%	<1.0
15-614 ***	-	5.5-7.0	235-265	>75%	<1.0

FIO - sólo para información

**Resultado de la prueba funcional

***Contenido de humedad #1.0

Almacenamiento

2°C hasta 8°C

Declaración de uso del producto

ESTOS PRODUCTOS SON PARA USO EXCLUSIVO EN INVESTIGACIÓN. No aprobado para uso humano o veterinario, para su aplicación en humanos o animales, ni para su uso en procedimientos clínicos o in vitro.

Lonza

Información de pedido

Catálogo número	Descripción	Tamaño
12-604F	DMEM con 4.5 g/L de glucosa, con L-glutamina	500 ml
12-604Q	DMEM con 4.5 g/L de glucosa, con L-glutamina	1 L
12-614F	DMEM con 4.5 g/L de glucosa, sin L-glutamina	500 ml
12-614Q	DMEM con 4.5 g/L de glucosa, sin L-glutamina	1 L
12-707F	DMEM con 1.0 g/L de glucosa, sin L-glutamina	500 ml
12-708F	DMEM con 1.0 g/L de glucosa, y 25 mM de agente amortiguador de HEPES, sin L-glutamina	500 ml
12-709F	DMEM con 4,5 g/L de glucosa y 25 mM de agente amortiguador de HEPES, sin L-glutamina	500 ml
12-733F	DMEM con 4.5 g/L de glucosa, sin L-glutamina, sin piruvato de sodio	500 ml
12-733Q	DMEM con 4.5 g/L de glucosa, sin L-glutamina, sin piruvato de sodio	1 L
12-741F	DMEM con 4.5 g/L de glucosa, con L-glutamina, sin piruvato de sodio	500 ml
12-914F	DMEM con glucosa de 4.5 g/L, sin L-glutamina, examinado para apoyar el crecimiento del hibridoma.	500 ml
12-917F	DMEM con 4.5 g/L de glucosa, sin L-glutamina ni rojo fenol.	500 ml
15-604D	DMEM con 4.5 g/L de glucosa, con L-glutamina, sin piruvato sódico, en polvo	1 x 10L
15-604F	DMEM con 4.5 g/L de glucosa, sin L-glutamina o piruvato de sodio, en polvo	1 X 50L
15-614D	DMEM con 4.5 g/L de glucosa, sin L-glutamina o piruvato sódico, en polvo	1 x 10L

Fig. 2

Lonza Walkersville, Inc.
www.lonza.com
biotechserv@lonza.com
Servicio Técnico: 800-521-0390
Documento # TS 12-615-2 11 /08
Walkersville, MD 21793-0127 EUA

Medio F12 de Ham

Uso del producto

El Medio F12 de Ham es una mezcla de nutrientes diseñada para cultivar una amplia variedad de células de mamíferos e hibridomas cuando se usa con suero en combinación con hormonas y transferrina.

Especificaciones

Esterilidad	pH	Osmolalidad (mOsm)
Negativa	7.07-7.40	286-305

Generación de Crecimiento celular	Endotoxina (EU/ml)
>75% de control	FIO

Almacenamiento

2°C hasta 8°C

Declaración de uso del producto

ESTOS PRODUCTOS SON PARA USO EXCLUSIVO EN INVESTIGACIÓN. No aprobado para uso humano o veterinario, para aplicación en humanos o animales, ni para uso en procedimientos clínicos o in vitro.

Información de pedido

Catálogo número	Descripción	Tamaño
12-615F	Medio F12 de Ham con L-glutamina	500 ml

Fig. 3

www.lonza.com
Soporte científico de EUA: 800-521-0390
scientific.support@lonza.com
Soporte científico de la UE/ROW: +49-221-99199-400
scientific.support.eu@lonza.com
Documento TS-12-719-3 08/11

Medio DMEM: F12 (1:1)

Uso del producto

DMEM combinado con Hams F12 se ha ampliamente utilizado para demostrar el efecto de varias hormonas y factores de crecimiento en los tejidos diana.

Descripción

12-719 con L-glutamina, HEPES 15 mM y 3.151 g/L de glucosa

15-719 polvo - con L-glutamina, HEPES 15 mM y 3.151 g/L de glucosa.
El medio en polvo requiere la adición de 16.0 ml/L de $NaHCO_3$, solución al 7.5% o 1.2 g/L de $NaHCO_3$ en polvo.

04-687 con 3.151 g/L de glucosa, con L-glutamina, sin HEPES

Especificaciones

12-719	Esterilidad	pH	Osmolalidad
	Negativo	7.0-7.4	(mOsm) 286-356
15-719*	Negativo	4.5-6.5	260-290
04-687	Negativo	FIO	FIO
	Promoción del crecimiento celular	Endotoxina (UE/ml)	Humedad
12-719	> 75% del control	FIO	
15-719*	> 75% del control	< 1 UE/ml	< 2%
04-687	–	FIO	–

* El pH, la osmolalidad, la endotoxina y la humedad se realizan sin $NaHCO_3$; se adiciona $NaHCO_3$ para la prueba de crecimiento celular.

Información de pedido

Catálogo número	Descripción	Tamaño
12-719F	Medio F12 de Ham con L-glutamina	500 ml
12-719Q	DMEM: F12 con L-glutamina, HEPES 15 mM y 3.151 g/L de glucosa	1 L
15-719D	DMEM: Polvo de F12 - con L-glutamina, HEPES 15 mM, y 3.151 g/L de glucosa. El medio en polvo requiere la adición de 16.0 ml/L de $NaHCO_3$, solución al 7.5% o 1.2 g/L de $NaHCO_3$ en polvo.	1 x 10L
04-687Q	DMEM: F12 con 3.151 g/L de glucosa, con L-glutamina, sin HEPES	1 L

Almacenamiento

2°C hasta 8°C

Declaración de uso del producto

ESTOS PRODUCTOS SON PARA USO EXCLUSIVO EN INVESTIGACIÓN. No aprobado para uso humano o veterinario, para su aplicación en humanos o animales, ni para su uso en procedimientos clínicos o in vitro.

Fig. 4

Cascade Biologics[MR]
invitrogen cell culture

GIBCO
invitrogen cell culture

Medio 171, Medio 171PRF, y MEGS

Medio 171

M-171-500

500 ml

Medio 171PRF (Sin rojo de fenol)

Medio 171PRF (Sin rojo de fenol)

M-171PRF-500

500 ml

Descripción del producto
Medio 171 y Medio 171PRF son medios de cultivo de tejidos líquidos estériles propuestos para uso como un componente en un entorno de cultivo completo para el crecimiento de células epiteliales mamarias humanas normales. El medio 171 es un medio basal que contiene aminoácidos esenciales y no esenciales, vitaminas, otros compuestos orgánicos, oligominerales, y sales inorgánicas. El medio 171PRF es una versión sin rojo de fenol del medio 171. Estos medios no contienen antibióticos, antimicóticos, hormonas, factores de crecimiento, o proteínas. Estos medios son HEPES y bicarbonato amortiguado y están diseñados para el uso en una incubadora con una atmósfera de 5% de CO_2/95% de aire. Para soportar la siembra en placas y proliferación a largo plazo de células epiteliales mamarias humanas normales, estos medios se deben complementar con el Complemento de Crecimiento Epitelial Mamario (MEGS, cat. no S-015-5).

Uso propuesto
El medio 171 se propone para el uso en el cultivo de rutina de células epiteliales mamarias humanas normales. El medio 171PRF se propone para el uso por investigadores que deseen cultivar células epiteliales mamarias humanas normales en la ausencia de rojo de fenol. Cuando se complementan con MEGS, estos medios soportarán la siembra en placas y proliferación de células epiteliales mamarias humanas normales a densidades entre 2.5×10^3 células/cm^2 y 8×10^4 células/cm_2. ***Este producto es para uso exclusivo de investigación. No es para el uso en animales, humanos, o procedimientos de diagnóstico.***

Precaución: Si se manipulan inapropiadamente, algunos componentes de este producto pueden presentar un peligro para la salud. Tome las precauciones adecuadas cuando se manipule este producto, incluyendo el uso de gafas y ropa protectora. Deshágase del mismo apropiadamente.

Almacenamiento y Estabilidad
El medio 171 y el medio 171PRF se almacenan a 4° C en nuestra instalación y se envían a temperatura ambiente. Después de que se reciben, estos medios se deben almacenar a 4° C y no se deben congelar. **Protéjase de la luz.** Varios componentes de estos medios de cultivo de tejidos son lábiles a la luz, y recomendamos que los medios no se expongan a la luz durante períodos prolongados de tiempo. Si los medios se calientan antes del uso, no exceda los 37° C. Cuando se almacena en la oscuridad a 4° C, el producto es estable hasta la fecha de vencimiento en la etiqueta.

Preparación del medio 171 complementado

1. Descongelar un bote de MEGS. Tomar un bote del medio del almacenamiento en frío. Asegurarse de que las tapas de los recipientes están apretadas.
2. Girar suavemente el bote de complemento. Evitar salpicar el complemento en la tapa del bote o provocar que el complemento produzca espuma.
3. Limpiar el exterior de los recipientes con una solución desinfectante tal como isopropanol o etanol al 70%.
4. Utilizando la técnica estéril en una campana de cultivo de flujo laminar, transferir todo el contenido del bote de complemento al bote del medio.
5. Tapar herméticamente el bote de medio complementado y girar el contenido para asegurar una solución homogénea. Evitar provocar que el medio produzca espuma.

Almacenamiento y Estabilidad del medio 171 complementado
Una vez que el medio 171 o el medio 171PRF se han complementado con MEGS, el medio complementado se debe almacenar en la oscuridad a 4° C y no se debe congelar. Cuando se almacena en la oscuridad a 4°C, el medio complementado es estable durante 1 mes.

Referencias Seleccionadas

La formulación del medio 171 es con base en MCDB 170, con modificaciones.

Hammond <SL, Hammond RG, Stampfer MR; PNAS 81:54355439, 1984

Fig. 4 continuación

Cascade Biologics[MR]
invitrogen cell culture

GIBCO
invitrogen cell culture

MEGS
Complemento para el crecimiento epitelial mamario

Cat. no. S-015-5
5 ml

Descripción del producto

El Complemento de Crecimiento Epitelial Mamario (MEGS) es una solución estéril y concentrada (100X) propuesta para el uso como un componente en un entorno de cultivo completo para el crecimiento de células epiteliales mamarias humanas normales. Cada bote de 5 ml de MEGS contiene todos los factores de crecimiento, hormonas, y extractos de tejido necesarios para el cultivo de células epiteliales mamarias humanas normales y es la cantidad correcta de complemento para un bote de 500 ml de medio 171 o medio 171PRF. El MEGS es un complemento iónicamente equilibrado que contiene extracto de hipófisis bovina (BPE), insulina bovina, hidrocortisona y factor de crecimiento epidérmico humano recombinante. Cuando un bote de 500 ml de medio 171 o medio 171PRF se complementa con MEGS, las concentraciones finales de los componentes en el medio complementado son: BPE, 0.4% v/v; insulina bovina, 5 pg/ml; hidrocortisona, 0.5 pg/ml; y factor de crecimiento epidérmico humano recombinante, 3 ng/ml.

Uso propuesto

El MEGS se propone para el uso en conjunto con el medio 171 o el medio 171PRF para el cultivo de rutina libre de suero de células epiteliales mamarias humanas normales.

Este producto es para uso exclusivo en investigación. No para uso en animales, humanos, o procedimientos de diagnóstico.

Precaución: Si se manipulan inapropiadamente, algunos componentes de este producto pueden presentar un peligro para la salud. Tome las precauciones apropiadas cuando se manipule este producto, incluyendo el uso de gafas y ropa protectora. Deshágase del mismo correctamente.

Almacenamiento y Estabilidad

El MEGS se almacena a -20° C en nuestra instalación y se envía en hielo seco. Después de que se recibe, el producto se debe almacenar a -20° C en un congelador que no sea auto-descongelante. Cuando se almacena a -20° C, el producto es estable hasta la fecha de vencimiento mostrada en la etiqueta.

Después de un almacenamiento a largo plazo a -20° C, el MEGS puede contener una pequeña cantidad de precipitado. Este precipitado se forma a partir de material insoluble en frío en el componente BPE de MEGS y no afectará al rendimiento del producto.

Descongelación

Para descongelar, coloque el producto en un baño de agua a 37° C o durante la noche a 4° C. Si se descongela en un baño de agua, no deje el producto a 37° C después de que el producto se haya descongelado. Para obtener instrucciones sobre la adición de MEGS al medio 171, consulte las instrucciones que acompañan al medio basal.

Referencias Seleccionadas

La formulación de MEGS es con base en la complementación publicada del medio MCDB 170, con modificaciones.

Hammond SL, Hammond RG, Stampfer MR; PNAS 81:54355439, 1984.

Para uso exclusivo en investigación. No se propone para ningún uso terapéutico o diagnóstico animal o humano.

Para uso exclusivo en investigación.

Fig. 5

Lista de ingredientes de medio PTT6

Lista de composición de medio	Nombre de la Compañía	Número de Catálogo
Medios básicos		
DMEM (también conocido en la presente como como medio basal PTT6)	Lonza	12-604F
DMEM/F12	Lonza	12-719F
M171	Life technologies	M171500
Suero		
Suero fetal bovino	GE Healthcare	A15-151
Antibiotico		
Penicilina - Estreptomicina - Anfotericina B	Lonza	17-745E
Complementos		
Adenina (opcional)	Sigma	A8626-25G
Hidrocortisona (opcional)	Sigma	H-0888
Factor de crecimiento epidérmico	Millipore	GF-144
T3 (3,3',5-Triiodo-L- sal sódica de tironina) (opcional)	Sigma	200-223-5
Insulina humana recombinante AOF	Life Technologies	A11382IJ

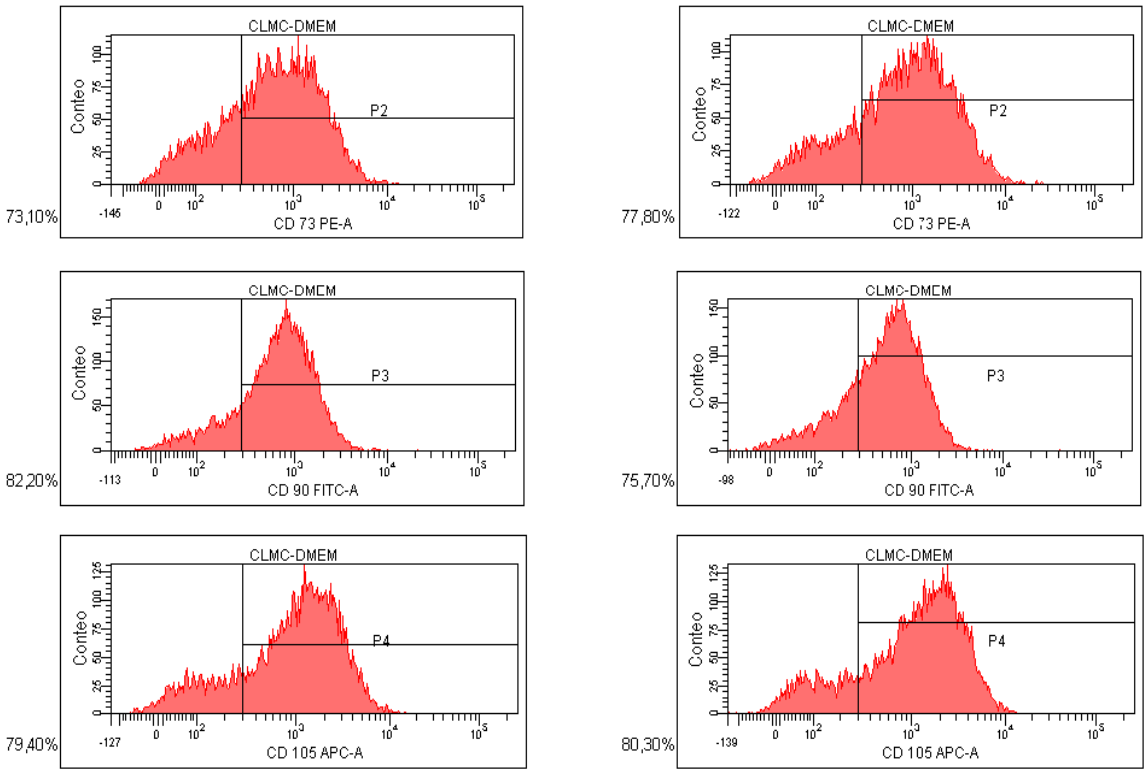

CLMC-DMEM
Conteo
P2
CD 73 PE-A
73,10%
CLMC-DMEM
Conteo
P2
CD 73 PE-A
77,80%
CLMC-DMEM
Conteo
P3
CD 90 FITC-A
82,20%
CLMC-DMEM
Conteo
P3
CD 90 FITC-A
75,70%
CLMC-DMEM
Conteo
P4
CD 105 APC-A
79,40%
CLMC-DMEM
Conteo
P4
CD 105 APC-A
80,30%

Fig. 6a

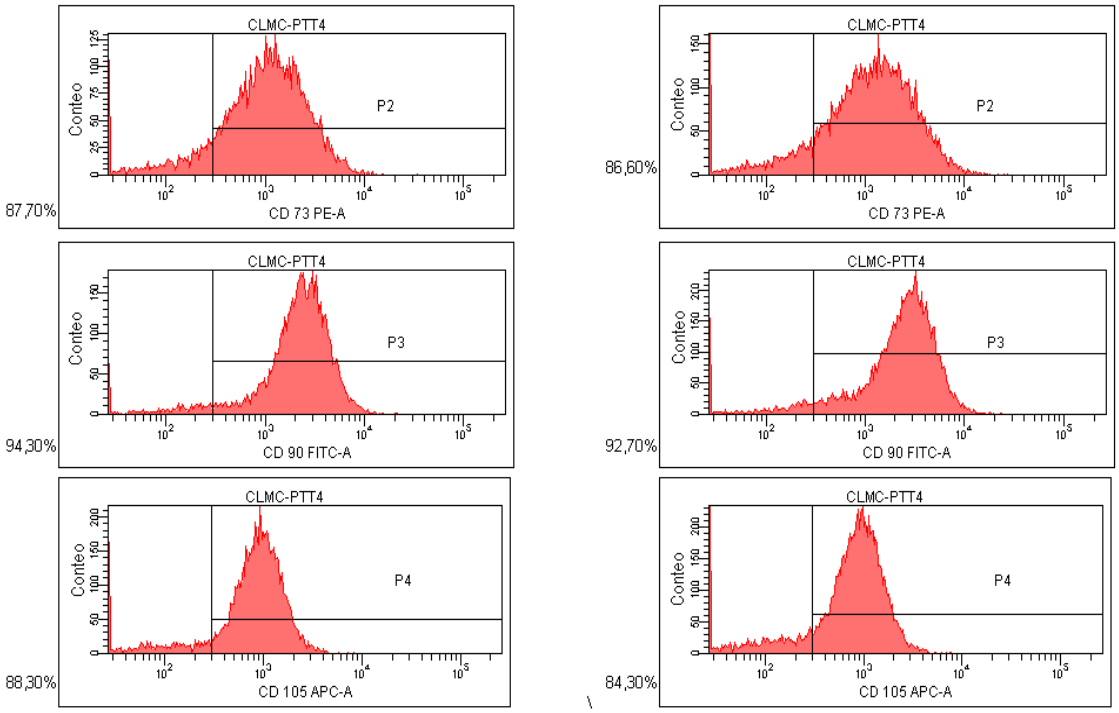

CLMC-PTT4
Conteo
P2
CD 73 PE-A
87,70%
CLMC-PTT4
Conteo
P2
CD 73 PE-A
86,60%
CLMC-PTT4
Conteo
P3
CD 90 FITC-A
94,30%
CLMC-PTT4
Conteo
P3
CD 90 FITC-A
92,70%
CLMC-PTT4
Conteo
P4
CD 105 APC-A
88,30%
CLMC-PTT4
Conteo
P4
CD 105 APC-A
84,30%

Fig. 6b

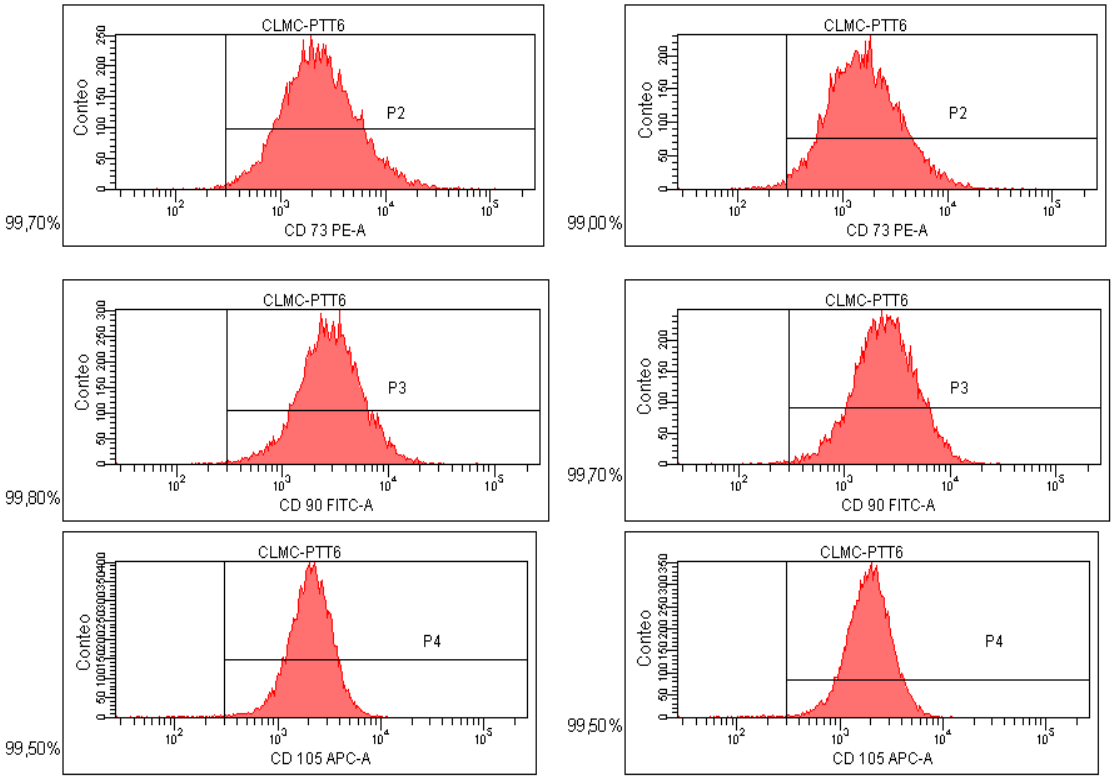
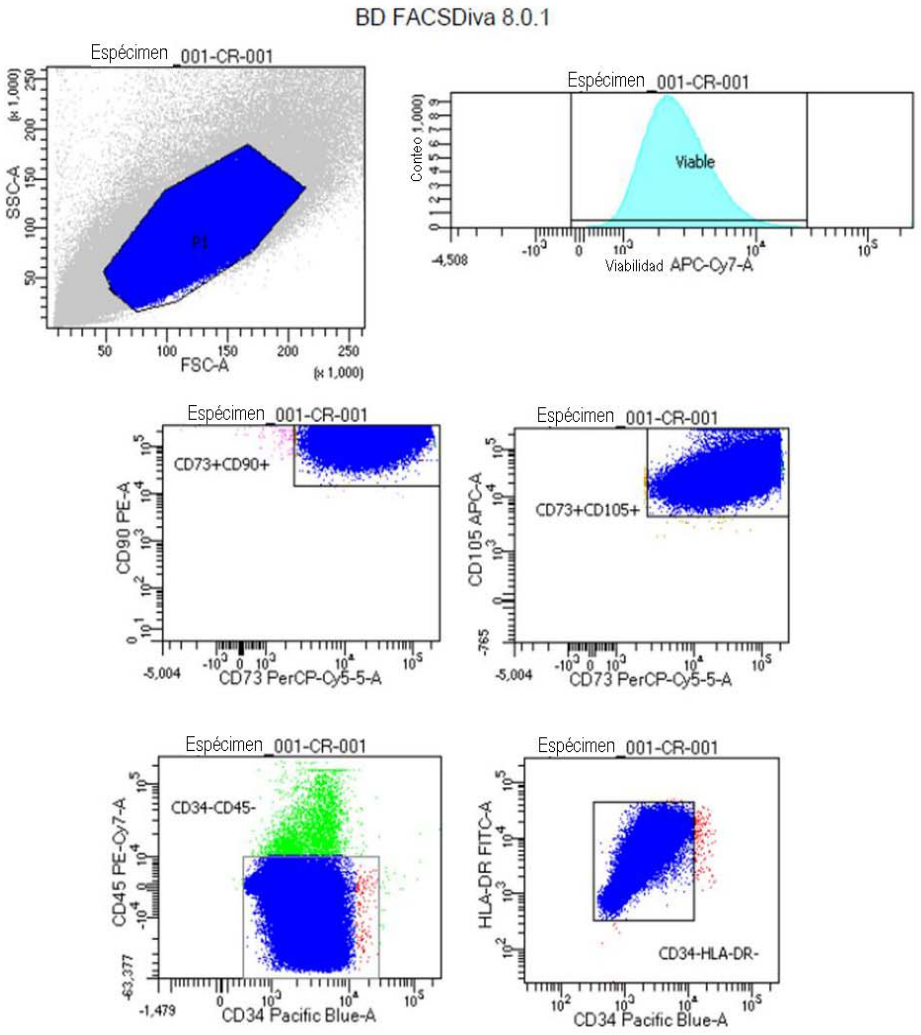

CLMC-PTT6
Conteo
P2
CD 73 PE-A
99,70%
CLMC-PTT6
Conteo
P2
CD 73 PE-A
99,00%
CLMC-PTT6
Conteo
P3
CD 90 FITC-A
99,80%
CLMC-PTT6
Conteo
P3
CD 90 FITC-A
99,70%
CLMC-PTT6
Conteo
P4
CD 105 APC-A
99,50%
CLMC-PTT6
Conteo
P4
CD 105 APC-A
99,50%

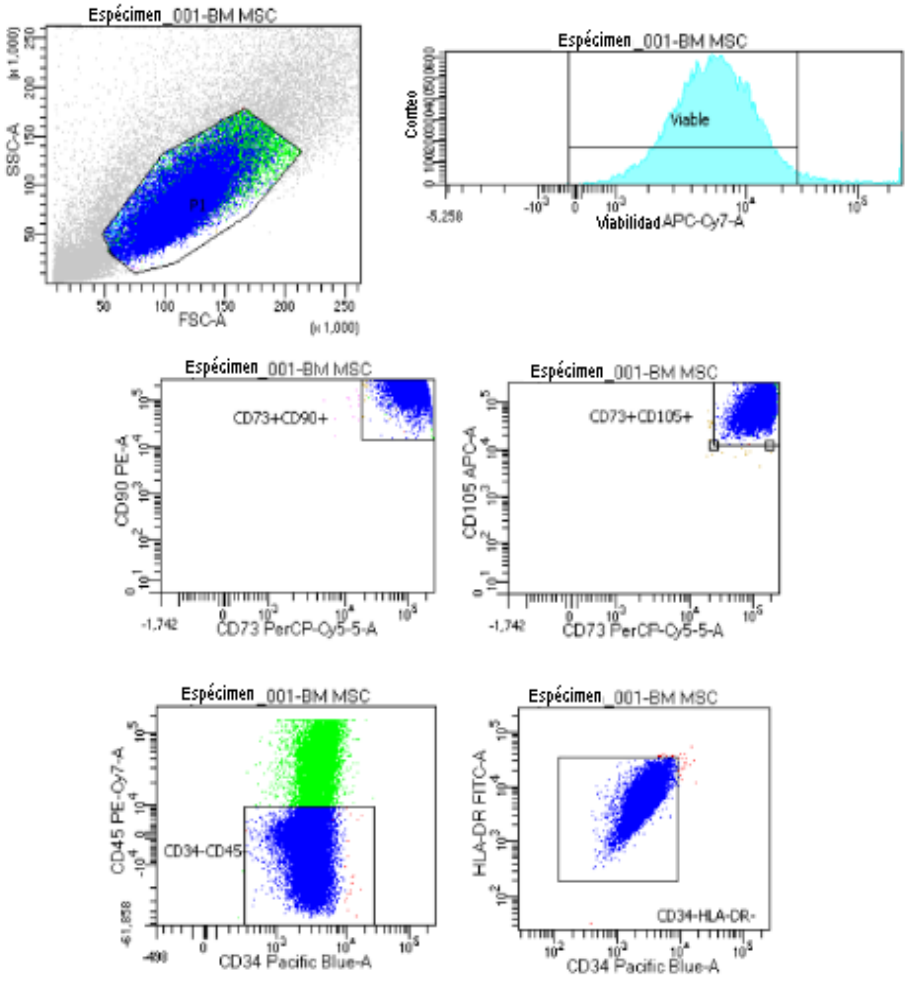
Fig. 6c



Tubo: CR-001

Población	#Eventos	% Original	%Total
Todos los Eventos	540,988	####	100.0
P1	425,093	78.6	78.6
Viable	414,643	97.5	76.6
CD73+CD90+	414,554	100.0	76.6
CD73+CD105+	414,490	100.0	76.6
CD34-CD45-	411,206	99.2	76.0
CD34-HLA-DR	411,061	100.0	76.0

Fig. 7a



Nombre de Experimento:	MSC 062216
Nombre de Espécimen:	Espécimen_001
Nombre de Tubo:	BM MSC

Población	#Eventos	% original
CD34-HLA-DR-	21,128	99.9

Población	#Eventos	% original	%Total
Todos los eventos	77,022	####	100.0
P1	35,780	46.5	46.5
Viable	33,741	94.3	43.8
CD73+CD90+	33,729	100.0	43.8
CD73+CD105+	33,710	99.9	43.8
CD34-CD45-	21,155	62.8	27.5
CD34-HLA-DR	21,128	99.9	27.4

Fig. 7b