MesenCult™-hPL Medium

For the Culture of Human Mesenchymal Stem Cells

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Catalog #05439 1 Kit

Product Description

MesenCult™-hPL Medium has been optimized for the culture and expansion of human mesenchymal stem and progenitor cells (MSCs) as well as for the detection and enumeration of colony-forming unit fibroblasts (CFU-F). MesenCult™-hPL 10X Supplement is an alternative growth supplement to fetal bovine serum (FBS) that contains purified human platelet lysate (hPL). Complete MesenCult™-hPL Medium (Basal Medium + 10X Supplement) does not require addition of growth factors, lipids, or attachment substrate. It is more defined, provides better performance than FBS-containing media, and is a more affordable option than defined, serum-free media.

Product Information

The following components are sold as a complete kit (Catalog #05439) and are not available for individual sale.

PRODUCT NAME	CATALOG #	SIZE	STORAGE	SHELF LIFE
MesenCult™-hPL Basal Medium*	05437	450 mL	Store at 2 - 8°C.	Stable for 12 months from date of manufacture (MFG) on label.
MesenCult™-hPL 10X Supplement**	05438	50 mL	Store at -20°C.	Stable until expiry date (EXP) on label.

*Medium stored for more than two months following the date of manufacture (MFG) on label should be supplemented with additional L-glutamine. For example, add 1 mL of 200 mM L-Glutamine (Catalog #07100) to 99 mL of medium to achieve a final concentration of 2 mM.
**This supplement has been pretested and selected for the ability to maintain and expand human MSCs in culture, and to sustain colony formation using the CFU-F assay. This supplement contains fibrinogen-depleted human platelet lysate and does not require the addition of anticoagulants. Please refer to the Safety Data Sheet for hazard information. This product contains material derived from human plasma. Donors have been tested and found negative for HIV-1 and -2, hepatitis B, and hepatitis C prior to donation. However, this product should be considered potentially infectious and treated in accordance with universal handling precautions.

None of the above components contain antibiotics.

Preparation of Complete MesenCult™-hPL Medium

Use sterile techniques to prepare complete MesenCult™-hPL Medium (Basal Medium + 10X Supplement). The following example is for preparing 500 mL of complete medium. If preparing other volumes, adjust accordingly.

1. Thaw MesenCult™-hPL 10X Supplement at room temperature (15 - 25°C) or at 2 - 8°C overnight. Mix thoroughly.

   NOTE: Once thawed, use immediately or aliquot and store at -20°C until expiry date (EXP) on label. After thawing the aliquoted supplements, use immediately. Do not re-freeze.
2. Add 50 mL of MesenCult™-hPL 10X Supplement to 450 mL of MesenCult™-hPL Basal Medium. Mix thoroughly.
3. Filter sterilize complete medium using a 0.22 µm filter.

   NOTE: If not used immediately, store complete MesenCult™-hPL Medium at 2 - 8°C for up to 2 weeks. Do not exceed the shelf life of the individual components.

Directions for Use

Please read the entire protocol before proceeding.

Use sterile techniques when performing the following protocols.

A) Isolation of Human MSCs from Bone Marrow (BM)

B) CFU-F Assay

C) Expansion of Human MSCs

NOTE: Enriched MSC samples can be obtained from human BM using either the RosetteSep™ Human Mesenchymal Stem Cell Enrichment Cocktail (Catalog #15128) or EasySep™ Human CD271 Selection Kit (Catalog #18659).

NOTE: For detailed instructions on how to isolate human MSCs from adipose tissue, refer to the Technical Bulletin: Culture Adipose-Derived Mesenchymal Stem Cells (Document #29108), available on our website at www.stemcell.com or contact us to request a copy.

A) Isolation of Human MSCs from Bone Marrow (BM)

The following protocol is for isolating MSCs from 25 mL of freshly isolated human BM using density gradient centrifugation with Lymphoprep™ (Catalog #07801). If using other volumes, adjust accordingly.

1. Count nucleated cells in the BM sample using 3% Acetic Acid with Methylene Blue (Catalog #07060). Refer to the Product Information Sheet (Document #29604) for directions for use.
2. Split the BM sample into two 50 mL tubes (i.e. 12.5 mL of BM sample per tube).
3. Add 22.5 mL of phosphate-buffered saline (PBS) containing 2 mM EDTA per tube.
4. Prepare three new 50 mL tubes and add 17 mL of Lymphoprep™ to each tube.
5. Carefully layer 23 mL of the BM suspension (from step 3) on top of the Lymphoprep™ in each tube.
6. Centrifuge tubes at 300 x *g* for 30 minutes, with the **brake off**.
7. Collect the mononuclear cell (MNC) layer, at the plasma:Lymphoprep™ interface, and place in a single new 50 mL tube.

   NOTE: Sometimes it is difficult to see the cells at the interface. In this case, it is recommended to remove some of the Lymphoprep™ along with the enriched cells in order to maximize cell recovery.
8. Wash cells with PBS containing 2 mM EDTA.
9. Centrifuge the tube at 300 x *g* for 10 minutes with the **brake on**.
10. Discard the supernatant and resuspend the cell pellet in complete MesenCult™-hPL Medium.
11. Count nucleated cells using 3% Acetic Acid with Methylene Blue.

B) CFU-F Assay

NOTE: The CFU-F assay cannot be performed with previously frozen BM MNCs.

The following protocol is for setting up a CFU-F assay in a 6-well plate. If using other cultureware, adjust cell numbers and volumes accordingly.

NOTE: Only use tissue culture-treated cultureware.

1. Plate cells (isolated in section A) in 2 mL of complete MesenCult™-hPL Medium per well. Plate cells at 3 different densities for each cell type used. Refer to Table 1 for recommended cell plating densities.

Table 1: Recommended Cell Plating Densities for Setting up the CFU-F Assay

CELL TYPE	CELLS PER CM^2	EXAMPLE OF CELL DENSITIES PER WELL OF A 6-WELL PLATE
BM-derived MSCs	2 - 10 x 10^4	2.5 x 10^5 5 x 10^5 7.5 x 10^5
Adipose-derived MSCs	0.5 - 5 x 10^3	0.5 x 10^4 2.5 x 10^4 5 x 10^4
RosetteSep™ enriched BM-derived MSCs	0.5 - 2 x 10^3	5 x 10^3 1 x 10^4 2 x 10^4
EasySep™ enriched BM-derived MSCs	1.5 - 10 x 10^3	2 x 10^4 5 x 10^4 10 x 10^4

2. Incubate cells at 37°C for 10 - 15 days until colonies (> 40 cells/colony) appear in the well.
3. Fix, stain and count the CFU-F colonies.

C) Expansion of Human MSCs

The following protocol is for culturing cells in a single T-75 cm^2 flask. If using other cultureware, adjust cell numbers and volumes accordingly.

NOTE: Only use tissue culture-treated cultureware.

1. Plate freshly isolated MSCs in 14 mL of complete MesenCult™-hPL Medium per flask. Refer to Table 2 for recommended cell plating densities of freshly isolated primary cells.

Table 2: Recommended Cell Plating Densities for Cell Expansion of Freshly Isolated Cells

CELL TYPE	FRESHLY ISOLATED CELLS PER CM^2	EXAMPLE OF CELL DENSITIES PER T-75 CM^2 FLASK
BM-derived MSCs	4 - 10 x 10^4	3.5 - 6 x 10^6
Adipose-derived MSCs	2.5 - 10 x 10^3	2 - 6 x 10^5
RosetteSep™ enriched BM-derived MSCs	4 - 10 x 10^3	3.5 - 6 x 10^5
EasySep™ enriched BM-derived MSCs	4 - 10 x 10^3	3.5 - 6 x 10^5

2. Incubate cells at 37°C until cells are approximately 80% confluent. This takes approximately 10 - 15 days.

   NOTE: If needed, perform a half medium change on day 7 (i.e. aspirate 7 mL of medium and add 7 mL of complete MesenCult™-hPL Medium per flask).

3. Passage cells using the following protocol:
   i. Wash cells once with 4 mL of D-PBS Without Ca++ and Mg++ (Catalog #37350).
   ii. Add 4 mL of Trypsin-EDTA (0.25%; Catalog #07901) and incubate at 37°C for 5 minutes. Tap the flask to detach cells. If < 90% of cells have detached, incubate at 37°C for an additional 3 minutes and tap the flask again.
   iii. Add 4 mL of complete MesenCult™-hPL Medium and collect cells in a 15 mL tube.
   iv. Centrifuge the tube at 300 x *g* for 10 minutes.
   v. Discard the supernatant, resuspend the cell pellet in complete MesenCult™-hPL Medium, and count and plate cells according to Table 3.

Table 3: Recommended Cell Plating Densities for Cell Expansion of Passaged Cells

CELL TYPE	PASSAGED CELLS PER CM^2	EXAMPLE OF CELL DENSITIES PER T-75 CM^2 FLASK
BM-derived MSCs	1.5 - 3 x 10^3	1.5 - 2.5 x 10^5
Adipose-derived MSCs	1.5 - 3 x 10^3	1.5 - 2.5 x 10^5

4. Repeat steps 2 and 3 as needed.