MesenCult™ (Human)

For the culture of human mesenchymal stem and progenitor cells

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Product Description

The MesenCult™ (Human) products listed below (see Product Information) are for the culture of human mesenchymal stem and progenitor cells (MSCs). Complete MesenCult™ Medium (Basal Medium + Stimulatory Supplement) is serum-containing and has been optimized for the expansion of human MSCs in vitro as well as for the detection and enumeration of colony-forming unit-fibroblasts (CFU-F).

For a complete list of related products available from STEMCELL Technologies, including differentiation media, visit our website at www.stemcell.com or contact us at techsupport@stemcell.com.

The MesenCult™ Proliferation Kit (Human; Catalog #05411) includes MesenCult™ MSC Basal Medium (Human; 450 mL) and MesenCult™ MSC Stimulatory Supplement (Human; 50 mL).

Product Information

PRODUCT NAME	CATALOG #	SIZE	STORAGE	SHELF LIFE
MesenCult™ MSC Basal Medium (Human)*	05401	450 mL	Store at 2 - 8°C.	Stable for 12 months from date of manufacture (MFG) on label.
MesenCult™ MSC Stimulatory Supplement (Human)**	05402	50 mL	Store at -20°C.	Stable until expiry date (EXP) on label.

*Medium stored for more than 2 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.
**Contains proprietary supplements that have been pretested and selected for their ability to maintain and expand human MSCs in culture, and to sustain colony formation using the CFU-F assay.

None of the above components contain antibiotics.

Preparation of Complete MesenCult™ Medium (Human)

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

1. Thaw Stimulatory 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 supplement, use immediately. Do not re-freeze.

   NOTE: Precipitate may be observed in thawed supplement or in complete medium in culture over time. This will not affect performance of the medium. Centrifugation or filtration to remove precipitate is not recommended.
2. Add 50 mL of Stimulatory Supplement to 450 mL of Basal Medium. Mix thoroughly.

   NOTE: If not used immediately, store complete MesenCult™ Medium at 2 - 8°C for up to 1 month. 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 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 in Serum-Free, Xeno-Free MesenCult™-XF (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 Dulbecco's Phosphate Buffered Saline with 2% Fetal Bovine Serum (PBS + 2% FBS; Catalog #07905) and 1 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 + 2% FBS and 1 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™ Medium.
11. Count nucleated cells using 3% Acetic Acid with Methylene Blue.

B) CFU-F Assay

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

NOTE: Only use tissue culture-treated cultureware.

1. Plate cells (isolated in section A) in 2 mL of complete MesenCult™ 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™ 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™ 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 less than 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™ Medium and collect cells in a 15 mL tube.
   iv. Centrifuge the tube at 300 x *g* for 10 minutes.
   v. Discard the supernatant and resuspend the cell pellet in complete MesenCult™ Medium.
   vi. 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.