MesenCult™ Proliferation Kit (Human)

Medium for detection of CFU-F and expansion of human mesenchymal stem cells

Catalog #05411	1 Kit
Catalog #05401	450 mL
Catalog #05402	50 mL

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Product Description

MesenCult™ Proliferation Kit (Human) is for the culture of human mesenchymal stromal 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).

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

Product Information

The following products are components of MesenCult™ Proliferation Kit (Catalog #05411) and are also available for individual sale.

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.

*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) Isolation of Human MSCs from Umbilical Cord (Perivascular Wharton's Jelly; UC-MSCs)

C) Isolation of Human MSCs from Adipose Tissue (ADSCs)

D) CFU-F Assay

E) 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).

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 MNCs 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 MNCs using 3% Acetic Acid with Methylene Blue.

B) Isolation of Human MSCs from Umbilical Cord (Perivascular Wharton's Jelly; UC-MSCs)

1. In a 10 cm culture dish, cut a fresh whole umbilical cord (< 48 hours after birth) into ~5 cm pieces.
2. Carefully dissect out the vessels with surrounding Wharton's Jelly (WJ), avoiding cutting into the vessel (to reduce contaminating red blood cells and endothelial cells).
3. Add D-PBS (Without Ca++ and Mg++) (PBS; Catalog #37350) dropwise to tissue periodically while dissecting (~every 5 - 10 minutes); this will maintain moisture.
4. Discard the amnion and outer WJ.
5. Carefully strip the perivascular WJ from the dissected vessels and transfer to a 50 mL conical tube (e.g. Catalog #38010) containing a small amount of PBS (just enough to cover the tissue). Discard stripped vessels.

   NOTE: Perivascular WJ should peel away from the vessel in long strips.
6. Pour the perivascular WJ strips into a new 10 cm dish and mince into ~3 mm^2 pieces, adding PBS periodically.
7. Add 8 mL of complete MesenCult™ Medium to each of ~20 x T-75 cm^2 flasks and incubate at 37°C for 30 minutes.

   NOTE: A volume of 8 mL of medium per T-75 cm^2 flask is recommended in order to maximize contact of the tissue with the flask for increased attachment success. Approximately 10 - 30 flasks will be required, depending on the size of the umbilical cord and the desired plating density.
8. Add minced WJ pieces to a fresh 50 mL conical tube containing enough PBS or MesenCult™ Medium to cover tissue. Store on ice.
9. Using a wide-bore pipette, add 1 - 2 mL of minced WJ pieces to each T-75 cm^2 flask (prepared in step 7).
10. Incubate at 37°C for 5 - 6 days, undisturbed, before checking for growth. This allows the tissue pieces to attach to the flask surface.
11. Perform a half-medium change on day 6 or day 7 as follows:
    a. Carefully remove 4 mL of medium. Do not disturb the attached explants. If some are floating, they may be removed, but it is best to avoid them.
    b. Slowly add 5 mL of warmed fresh medium.
    c. Incubate at 37°C.
12. At day 10 - 14, many of the explants should have balled up and detached. This is an indicator that the cells (migrated out from the explants) are ready for harvest. Also, if the colonies formed are beginning to detach or peel, the cells are ready for harvest. Proceed to step 13.

<u>Harvesting/Passaging UC-MSCs</u>

The following protocol is for harvesting/passaging UC-MSCs using Animal Component-Free Cell Dissociation Kit (Catalog #05426).

13. Using a wide-bore pipette, carefully remove and discard medium and loose fragments.
14. Carefully rinse flask 2X with PBS.
15. Add 5 mL of ACF Enzymatic Dissociation Solution. Incubate at 37°C for 6 - 7 minutes.

16. Tap the bottom and sides of the flask to detach cells. If 10 - 20% of cells have not detached, incubate for an additional 2 - 3 minutes.

    NOTE: Do not pipette up and down to release cells. If cells are not detached after 9 minutes of dissociation, add 5 mL of ACF Enzyme Inhibition Solution. Transfer detached cells to a 50 mL conical tube, then add 5 mL of complete MesenCult™ Medium to the flask. Use a cell scraper to carefully release the remaining cells and transfer cells to the conical tube. Proceed to step 19.
17. Add 5 mL of ACF Enzyme Inhibition Solution.
18. Transfer detached cells to a 50 mL conical tube (do not pipette up and down).
19. Rinse the flask 2 - 3X with complete MesenCult™ Medium; add the rinses (containing remaining cells and medium) to the conical tube.
20. Filter cells through a 100 µm Reversible Strainer (Catalog #27270) into a new 50 mL conical tube.
21. Centrifuge at 285 x *g* for 10 minutes.
22. Aspirate supernatant, then flick the tube to resuspend pellet (do not pipette up and down).
23. Perform a viable cell count using Trypan Blue (Catalog #07050).
24. Plate cells at a density of 1 - 3 x 10^3 cells/cm^2 for expansion; refer to Table 1 for recommended number of cells for various cultureware.

    NOTE: Do not freeze cells at the end of P0, as they are fragile and this will result in poor recovery post-thaw. If needed, freeze cells after P1.

Table 1. Recommended Number of Cells for Various Cultureware

TISSUE CULTURE-TREATED CULTUREWARE	NUMBER OF CELLS TO PLATE*	VOLUME OF MEDIUM
6-well plate (e.g. Catalog #38016)	1 - 3 x 10^4 cells/well	2 mL/well
T-25 cm^2 flask	2.5 - 7.5 x 10^4 cells/flask	5 mL/flask
T-75 cm^2 flask	0.75 - 2.25 x 10^5 cells/flask	10 - 12 mL/flask

*It is recommended to plate two different densities within the range provided (i.e. 1.5 x 10^4 and 3 x 10^4 cells/well in a 6-well plate).

C) Isolation of Human MSCs from Adipose Tissue (ADSCs)

1. In a 50 mL conical tube, add 2 - 4 mL of 0.25% Collagenase Type I (0.25%; Catalog #07902) to the adipose tissue.
2. Finely mince tissue with a scalpel, then transfer to a 50 mL conical tube.
3. Add 5 mL of 0.25% Collagenase Type I per cm^3 of tissue. Incubate at 37°C for 1 hour in a shaking water bath or shaking incubator (e.g. for 3 cm^3 tissue use 15 mL Collagenase Type I).
4. Place the samples upright for 5 minutes to allow separation of the lipid layer from the aqueous layer.
5. Using a serological pipette or an aspirator, remove and discard the top lipid layer.
6. Add PBS + 2% FBS and 1 mM EDTA to reach a final volume of 50 mL.
7. Centrifuge at 300 x *g* for 10 minutes with the brake on.
8. Remove and discard supernatant. Resuspend the cell pellet in complete MesenCult™ Medium.
9. Count nucleated cells using 3% Acetic Acid with Methylene Blue (Catalog #07060).

D) 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 2 for recommended cell plating densities.

Table 2: Recommended Cell Plating Densities for Setting up the CFU-F Assay

CELL TYPE	CELLS PER cm^2	EXAMPLE OF NUMBER OF CELLS 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.

E) 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 MNCs in 14 mL of complete MesenCult™ Medium per flask. Refer to Table 3 for recommended cell plating densities of freshly isolated primary cells.

Table 3: Recommended Cell Plating Densities for Expansion of Freshly Isolated Cells

CELL TYPE	FRESHLY ISOLATED CELLS PER cm^2	EXAMPLE OF NUMBER OF CELLS PER T-75 cm^2 FLASK
BM-derived MNCs	4 - 10 x 10^4	3.5 - 6 x 10^6
Adipose-derived MNCs	2.5 - 10 x 10^3	2 - 6 x 10^5
RosetteSep™-enriched BM-derived MNCs	4 - 10 x 10^3	3.5 - 6 x 10^5
EasySep™-enriched BM-derived MNCs	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 as follows:

   For UC-MSCs (use Animal Component-Free Cell Dissociation Kit [Catalog #05426]):
   i. Wash cells once with 4 mL of D-PBS (Without Ca++ and Mg++; Catalog #37350).
   ii. Add 6 mL of ACF Enzymatic Dissociation Solution.
   iii. Incubate at 37°C for 5 - 8 minutes. Tap the flask to detach cells. If < 90% of cells have detached, incubate at 37°C for an additional 1 - 2 minutes and tap the flask again.
   iv. Add 6 mL of ACF Enzyme Inhibition Solution. Transfer cells to a 50 mL conical tube (e.g. Catalog #38010).
   v. Wash the flask with 4 - 6 mL of complete MesenCult™ Medium. Transfer wash to the tube containing cells. Proceed to step 4.

   OR

   For BM- and adipose-derived MSCs:
   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)
   iii. 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.
   iv. Add 4 mL of complete MesenCult™ Medium to the flask. Transfer cells to a 15 mL conical tube (e.g. Catalog #38009).
4. Centrifuge at 300 x *g* for 8 minutes.
5. Discard the supernatant and resuspend the cell pellet in complete MesenCult™ Medium.

6. Count and plate cells according to Table 4.

Table 4: Recommended Cell Plating Densities for Expansion of Passaged Cells

CELL TYPE	PASSAGED CELLS PER cm^2	EXAMPLE OF NUMBER OF CELLS 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
Umbilical cord-derived MSCs	1.5 - 3 x 10^3	1.5 - 2.5 x 10^5

7. Repeat steps 2 - 6 as needed.

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