MesenCult™ (Mouse)

For the culture of mouse MSCs and MEFs

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Product Description

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

To facilitate the enrichment of MSCs and MEFs during cell culture without serial passaging and frequent medium changes, simply add MesenPure™ to complete MesenCult™ Medium just prior to use. Although not required, the addition of MesenPure™ is strongly recommended, as resulting MSC and MEF cultures are more homogeneous and exhibit more robust proliferation, differentiation, and colony formation when compared to complete MesenCult™ Medium alone.

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 with MesenPure™ (Mouse; Catalog #05512) includes MesenCult™ MSC Basal Medium (Mouse; 400 mL), MesenCult™ MSC Stimulatory Supplement (Mouse; 100 mL), and MesenPure™ (0.5 mL).

Product Information

PRODUCT NAME	CATALOG #	SIZE	STORAGE	SHELF LIFE
MesenCult™ MSC Basal Medium (Mouse)*	05501	400 mL	Store at 2 - 8°C.	Stable for 12 months from date of manufacture (MFG) on label.
MesenCult™ MSC Stimulatory Supplement (Mouse)	05502	100 mL	Store at -20°C.	Stable until expiry date (EXP) on label.
MesenPure™ 1000X**	05500	0.5 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.
**Please refer to the Safety Data Sheet (SDS) for hazard information. This product contains components dissolved in dimethyl sulfoxide (DMSO). DMSO is a strong solvent and skin penetrant, and can transport many substances through the skin. DMSO can also penetrate some protective glove materials including latex and silicone. Extra caution should be used when handling this product. This component is sold as part of the MesenCult™ Proliferation Kit with MesenPure™ (Mouse; Catalog #05512) and is not available for individual sale.

None of the above components contain antibiotics.

Preparation of Complete MesenCult™ Medium (Mouse)

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 100 mL of Stimulatory Supplement to 400 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.

   OPTIONAL: Add MesenPure™ for setting up the CFU-F assay or for expanding MSCs or MEFs (see the next section).

MesenPure™ Addition (Recommended)

Although not required, the addition of MesenPure™ to complete MesenCult™ Medium is strongly recommended, as the resulting MSC and MEF cultures are more homogeneous and exhibit more robust proliferation, differentiation, and colony formation when compared to complete MesenCult™ Medium alone.

1. Thaw MesenPure™ at room temperature (15 - 25°C). Mix thoroughly.

   NOTE: Once thawed, use immediately or aliquot and store at -20°C until expiry date (EXP) on label. After thawing the aliquoted MesenPure™, use immediately. Do not re-freeze.
2. Dilute MesenPure™ 1 in 1000 in complete MesenCult™ Medium (e.g. add 1 µL of MesenPure™ per 1 mL of complete medium) and mix thoroughly.

   NOTE: Always add MesenPure™ to complete MesenCult™ Medium immediately prior to use. Do not store complete MesenCult™ Medium containing MesenPure™.

Directions for Use

Please read the entire protocol before proceeding.

For optimal results, culture cells under hypoxic conditions (5% O_2 and 5 - 10% CO_2) at 37°C in a humidified cell culture incubator or use a Hypoxia Incubator Chamber (Catalog #27310). For instructions on how to use the Hypoxia Incubator Chamber refer to the Product Information Sheet (PIS; Document #29829) available on our website at www.stemcell.com.

Use sterile techniques when performing the following protocols.

A) Isolation of Mouse MSCs from Compact Bone and Bone Marrow

B) CFU-F Assay

C) Expansion of Mouse MSCs and MEFs

NOTE: For detailed instructions on how to isolate mouse MSCs from adipose tissue or MEFs contact us at techsupport@stemcell.com.

A) Isolation of Mouse MSCs from Compact Bone (CB) and Bone Marrow (BM)

NOTE: MesenPure™ is not required in the following isolation protocols.

1. Sacrifice mice using procedures approved by your institution and remove the femurs and tibias from each mouse. Ensure that the bones are free of skin and muscle tissues.
2. Use a scalpel to cut the ends of the bones (epiphyses) to expose the interior of the marrow shaft. Place bones into a sterile mortar.
3. Add 10 mL of phosphate-buffered saline containing 2% fetal bovine serum (PBS + 2% FBS; Catalog #07905) and 1 mM EDTA to the mortar, and use a pestle to lightly crush the bones to release the marrow.

   NOTE: It is important to use gentle pressure when cracking the bones to release the marrow. Harsh grinding could result in unwanted cell death and excess debris.
4. Swirl the marrow suspension and collect it in a 50 mL tube.
5. Repeat steps 3 and 4 until all marrow has been released (i.e. the suspension is clear) and collect into the same 50 mL tube.

To obtain CB-derived MSCs follow steps 6 - 14; to obtain BM-derived MSCs follow steps 15 - 17.

CB-derived MSCs:

6. Transfer the bone fragments (from step 5) to a 100 mm dish containing 2 mL of Collagenase Type I (0.25%; Catalog #07902). Incubate at room temperature (15 - 25°C) for 3 - 4 minutes.
7. Use a scalpel to further cut the bone fragments into 1 - 2 mm pieces.
8. Transfer the bone suspension into a 50 mL tube and add Collagenase Type I (0.25%) to reach a final volume of 2 mL per mouse, or a minimum of 10 mL.
9. Incubate the tightly capped tube at 37°C for 45 minutes, on its side, while shaking at approximately 200 rpm.
10. Add PBS containing 2% FBS and 1 mM EDTA to the tube to achieve a final volume of 30 mL.
11. Filter the bone suspension through a 70 µm filter into a new 50 mL tube.
12. Wash the filter with 10 mL of PBS + 2% FBS and 1 mM EDTA and collect the wash into the same tube as in step 11.
13. Centrifuge the tube at 300 x g for 10 minutes.
14. Discard the supernatant and resuspend the cell pellet in complete MesenCult™ Medium.

BM-derived MSCs:

15. Filter the BM suspension (from step 5) through a 70 µm filter into a new 50 mL tube.
16. Centrifuge the tube at 300 x *g* for 10 minutes.
17. Discard the supernatant and resuspend the cell pellet in complete MesenCult™ Medium.

Expected Number of Total Cells Recovered After CB and BM Isolation

Following isolation, count nucleated cells using 3% Acetic Acid with Methylene Blue (Catalog #07060). Refer to the PIS (Document #29604) for directions for use. See Table 1 for expected cell numbers.

Table 1: Expected Number of Total Cells Recovered After Isolation

SOURCE	EXPECTED NUMBER OF TOTAL CELLS RECOVERED PER MOUSE (i.e. TWO FEMURS AND TWO TIBIAS)
CB	1.5 - 3.5 x 10^6
BM	3 - 5 x 10^7

NOTE: For additional protocol information, refer to the instructional videos available on our website at www.stemcell.com:

- Isolation of MSCs from Mouse Compact Bone
- How to Perform Cell Counts with a Hemocytometer

B) CFU-F Assay

NOTE: If using MesenPure™ to facilitate the enrichment of MSCs and MEFs, dilute MesenPure™ 1000X in complete MesenCult™ Medium just prior to use. Mix thoroughly and then plate cells. Do not store complete MesenCult™ Medium containing MesenPure™. Refer to the MesenPure™ Addition section for complete instructions.

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 in 2 mL of complete MesenCult™ Medium (with or without MesenPure™) 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 CELL DENSITIES PER WELL OF A 6-WELL PLATE
CB-derived MSCs	0.5 - 2.5 x 10^4	5 x 10^4 10 x 10^4 25 x 10^4
BM-derived MSCs	0.25 - 1 x 10^5	2.5 x 10^5 5 x 10^5 10 x 10^5
Adipose-derived MSCs	0.5 - 2.5 x 10^4	5 x 10^4 10 x 10^4 25 x 10^4
MEFs	0.5 - 2.5 x 10^3	5 x 10^3 10 x 10^3 25 x 10^3

2. Incubate cells at 37°C under hypoxic conditions for 7 days.
3. Perform a half-medium change (i.e. aspirate 1 mL of medium and add 1 mL of complete MesenCult™ Medium [with or without MesenPure™] per well).
4. Incubate cells at 37°C in hypoxic conditions until colonies (> 20 cells/colony) appear in the well. This takes approximately an additional 3 - 7 days (i.e. 10 - 14 days in total).
5. Fix, stain, and count the CFU-F colonies.

C) Expansion of Mouse MSCs and MEFs

NOTE: If using MesenPure™ to facilitate the enrichment of MSCs and MEFs, dilute MesenPure™ 1 in 1000 in complete MesenCult™ Medium just prior to use. Mix thoroughly and then plate cells. Do not store complete MesenCult™ Medium containing MesenPure™. Refer to the section on MesenPure™ addition for complete instructions.

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 or MEFs in 10 mL of complete MesenCult™ Medium (with or without MesenPure™) per flask. Refer to Table 3 for recommended cell plating densities of freshly isolated primary cells.

Table 3: 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
CB-derived MSCs	3 - 6 x 10^4	2.5 - 5 x 10^6
BM-derived MSCs	3 - 6 x 10^5	25 - 50 x 10^6
Adipose-derived MSCs	3 - 6 x 10^4	2.5 - 5 x 10^6
MEFs	3 - 6 x 10^3	2.5 - 5 x 10^5

2. Incubate cells at 37°C under hypoxic conditions for 7 days.
3. Perform a half-medium change (i.e. aspirate 5 mL of medium and add 5 mL of complete MesenCult™ medium [with or without MesenPure™] per flask).
4. Incubate cells at 37°C in hypoxic conditions until cells are approximately 80% confluent. This takes approximately an additional 3 - 7 days (i.e. 10 - 14 days in total).

   NOTE: When plating freshly isolated cells do not culture for more than 14 days and do not exceed 80% confluence.
5. 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 2 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 (with or without MesenPure™) according to Table 4.

Table 4: 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
CB-derived MSCs	6.7 - 10 X 10^3	5 - 7.5 x 10^5
BM-derived MSCs	10 - 13 x 10^3	7.5 - 10 x 10^5
Adipose-derived MSCs	3.3 - 6.7 x 10^3	2.5 - 5 x 10^5
MEFs	3.3 - 6.7 x 10^3	2.5 - 5 x 10^5

6. Repeat steps 4 and 5 as needed.