MesenCult™ Expansion Kit (Mouse)

For the culture of mouse MSCs and MEFs

Catalog #05513 1 Kit

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Product Description

MesenCult™ Expansion Kit (Mouse) is standardized for the culture of mouse mesenchymal stem and progenitor cells (MSCs) and mouse embryonic fibroblasts (MEFs). The kit includes MesenCult™ Basal Medium (Mouse), MesenCult™ 10X Supplement (Mouse), and MesenPure™. MesenCult™ Expansion Medium has been optimized for the derivation and expansion of mouse MSCs and MEFs in vitro as well as for the detection of colony-forming unit–fibroblasts (CFU-F). This kit was optimized using cells from the mouse strain C57BL/6.

To facilitate the enrichment of MSCs and MEFs during cell culture without serial passaging and frequent medium changes, simply add MesenPure™ to complete MesenCult™ Expansion Medium just prior to use. Although not required, the addition of MesenPure™ 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™ Expansion Medium alone.

NOTE: MesenCult™ Expansion Medium must be supplemented with L-Glutamine (Catalog #07100).

Product Information

The following components are sold as a complete kit (Catalog #05513) and are not available for individual sale.

COMPONENT NAME	COMPONENT #	SIZE	STORAGE	SHELF LIFE
MesenCult™ Basal Medium (Mouse)	05514	450 mL	Store at 2 - 8°C.	Stable for 12 months from date of manufacture (MFG) on label.
MesenCult™ 10X Supplement (Mouse)	05515	50 mL	Store at -20°C.	Stable for 24 months from date of manufacture (MFG) on label.
MesenPure™*	05500	0.5 mL	Store at -20°C.	Stable until expiry date (EXP) on label.

*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.

None of the above components contain antibiotics.

Preparation of Complete MesenCult™ Expansion Medium (Mouse)

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

1. Thaw 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. Do not exceed the shelf life of the supplement. After thawing the aliquots, use immediately. Do not re-freeze.
2. Add 50 mL of 10X Supplement to 450 mL of Basal Medium.
3. Add 5 mL of L-Glutamine to achieve a final concentration of 2 mM. Mix thoroughly.

   NOTE: If not used immediately, store complete MesenCult™ Expansion Medium at 2 - 8°C for up to 2 weeks. 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 next section).

MesenPure™ Addition (Recommended)

Although not required, the addition of MesenPure™ to complete MesenCult™ Expansion Medium is strongly recommended, as the resulting MSC and MEF cultures are more homogeneous and exhibit more robust proliferation, differentiation, and colony formation compared to cultures grown in the medium without MesenPure™. MesenPure™ facilitates the enrichment of MSCs and MEFs without serial passaging or frequent medium changes. MesenPure™ has been optimized using cells from the mouse strain C57BL/6. The efficacy of MesenPure™ on culture of cells from other mouse strains may vary.

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™ Expansion Medium (e.g. add 1 µL of MesenPure™ per 1 mL of complete medium) and mix thoroughly.

   NOTE: Always add MesenPure™ to complete MesenCult™ Expansion Medium immediately prior to use. Do not store complete MesenCult™ Expansion 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 at www.stemcell.com.

Use sterile techniques when performing the following protocols:

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

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. Prepare complete MesenCult™ Expansion Medium and warm to 37°C.
2. Sacrifice mice using procedures approved by your institution. Remove the femurs and tibias from each mouse. Ensure that the bones are free of skin and muscle tissue. Keep bones on ice in phosphate-buffered saline (PBS) containing 2% fetal bovine serum (FBS) and 1 mM EDTA (e.g. EasySep™ Buffer; Catalog #20144). Antibiotics may be added to the buffer during isolation if desired.
3. Use bone-cutting scissors to cut both ends of bones to expose the interior of the marrow shaft. Place bones in a sterile 100 mm dish containing 2% FBS and 1 mM EDTA on ice.
4. Using a 6 mL syringe with a 23 cc needle, draw up warm complete MesenCult™ Expansion Medium. Using sterile forceps, hold a bone over a 50 mL conical tube (e.g. Catalog #38010). Insert the needle into one end of the bone and gently push the syringe plunger to flush out the marrow with medium.
5. Repeat step 4 until all marrow has been flushed out of all bones (i.e. bones are white) and collect into the same 50 mL tube.
6. Resuspend the bone marrow by pipetting up and down until no clumps are observed.

To obtain CB-derived MSCs follow steps 7 - 15; to obtain BM-derived MSCs follow steps 16 - 17.

CB-derived MSCs:

7. Transfer the bones (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.
8. Use bone-cutting scissors to cut the bones into 1 - 2 mm fragments.
9. Transfer the bone suspension into a 50 mL conical tube. Add Collagenase Type I (0.25%) to reach a final volume of 2 mL per mouse, or a minimum of 10 mL.
10. Incubate the tightly capped tube at 37°C for 1 hour, on its side, while shaking at approximately 200 rpm.
11. Add PBS containing 2% FBS and 1 mM EDTA to the tube to achieve a final volume of 30 mL.
12. Filter the bone suspension through a 70 µm cell strainer into a new 50 mL tube.
13. Wash the strainer with 10 mL of PBS containing 2% FBS and 1 mM EDTA and collect the wash into the same tube as in step 12.
14. Centrifuge the tube at 300 x *g* for 10 minutes.
15. Discard the supernatant and resuspend the cell pellet in complete MesenCult™ Expansion Medium.

BM-derived MSCs:

16. Centrifuge the 50 mL tube containing bone marrow (from step 6) at 300 x *g* for 10 minutes.
17. Discard the supernatant. Resuspend the cell pellet in complete MesenCult™ Expansion Medium.

Expected Number of Total Cells Recovered After Isolation From CB and BM

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)
Compact bone (CB)	7 - 12 x 10^6
Bone marrow (BM)	5.5 - 6.5 x 10^7

B) CFU-F Assay

NOTE: If using MesenPure™ to facilitate the enrichment of MSCs and MEFs, add MesenPure™ to complete MesenCult™ Expansion Medium just prior to use. 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™ Expansion Medium (with or without MesenPure™) per well. Plate cells at 3 different densities for each cell type used in duplicate wells. Refer to Table 2 for recommended cell plating densities.

   NOTE: During the initial MSC isolation, antibiotics may be added to complete medium if desired.

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

CELL TYPE	RECOMMENDED PLATING DENSITY (cells/cm^2)	EXAMPLE OF NUMBER OF CELLS 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 10^4	5 x 10^4 10 x 10^4 25 x 10^4

2. Incubate cells at 37°C under hypoxic conditions until colonies (> 20 cells/colony) appear. This takes approximately 7 days.
3. If more time is needed, perform a half-medium change (i.e. aspirate 1 mL of medium and add 1 mL of complete MesenCult™ Expansion Medium [with or without MesenPure™] per well).
4. Incubate cells at 37°C under hypoxic conditions for an additional 2 - 7 days until ideal colony size is achieved.
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, add MesenPure™ to complete MesenCult™ Expansion Medium just prior to use. Refer to the MesenPure™ Addition section 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™ Expansion 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	RECOMMENDED PLATING DENSITY (freshly isolated cells/cm^2)	EXAMPLE OF NUMBER OF CELLS 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.

   NOTE: When plating freshly isolated cells, do not culture for more than 10 days and do not exceed 80% confluency.

   - If cells have reached 80% confluency, continue to step 3 to passage cells.

   OR

   - If cells are < 80% confluent:

   i. Perform a half-medium change (i.e. aspirate 5 mL of medium and add 5 mL of complete MesenCult™ Expansion Medium [with or without MesenPure™] per flask).

   ii. Incubate cells at 37°C under hypoxic conditions until cells have reached approximately 80% confluency. This takes approximately an additional 2 - 3 days (i.e. 9 - 10 days in total). Continue to step 3 to passage cells.

3. Passage cells as follows:

   i. Wash cells once with 5 mL of D-PBS (Without Ca++ and Mg++; Catalog #37350).

   ii. Add 5 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 5 mL of complete MesenCult™ Expansion Medium and collect cells in a 15 mL conical tube.

   iv. Centrifuge at 300 x *g* for 10 minutes.

   v. Discard the supernatant and resuspend the cell pellet in complete MesenCult™ Expansion Medium.

   vi. Count and plate cells (with or without MesenPure™) according to Table 4.

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

CELL TYPE	RECOMMENDED PLATING DENSITY (cells/cm^2)	EXAMPLE OF NUMBER OF CELLS PER T-75 cm^2 FLASK
CB-derived MSCs	4 - 6.7 X 10^3	3 - 5 x 10^5
BM-derived MSCs*	4 - 6.7 x 10^3	3 - 5 x 10^5
Adipose-derived MSCs	3.3 - 4.6 x 10^3	2.5 - 3.5 x 10^5
MEFs	3.3 - 4.6 x 10^3	2.5 - 3.5 x 10^5

*If MesenPure™ is not used in complete medium, seed 1.5 - 2 times more cells for BM-derived MSCs.

Related Products

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