EasySep™

Negative Selection

Catalog #19771

EasySep™ Mouse Mesenchymal Stem/Progenitor Cell Enrichment Kit

For processing 4 x 10^8 cells

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Description

Isolate untouched and highly purified mesenchymal stem/progenitor cells from mouse compact bone cell suspensions by immunomagnetic negative selection. When using single-cell suspensions from other tissue types, this kit may require optimization.

- Fast, easy-to-use and column-free
- Up to 99% purity
- Isolated cells are untouched

This kit targets non-mesenchymal progenitor cells for removal with biotinylated antibodies recognizing CD45 and TER119 cell surface markers. Unwanted cells are labeled with biotinylated antibodies and magnetic particles, and separated without columns using an EasySep™ magnet. Desired cells are simply poured off into a new tube. Isolated cells are immediately available for downstream applications such as flow cytometry, culture, or DNA/RNA extraction.

Component Descriptions

COMPONENT NAME	COMPONENT #	QUANTITY	STORAGE	SHELF LIFE	FORMAT
EasySep™ Mouse Mesenchymal Progenitor Enrichment Cocktail	19771C	1 x 0.4 mL	Store at 2 - 8°C. Do not freeze.	Stable until expiry date (EXP) on label.	A combination of monoclonal antibodies in PBS and 0.1% BSA.
EasySep™ Biotin Selection Cocktail	18153	2 x 1 mL	Store at 2 - 8°C. Do not freeze.	Stable until expiry date (EXP) on label.	A combination of monoclonal antibodies in PBS.
EasySep™ M Prog Magnetic Microparticles	19350	2 x 1 mL	Store at 2 - 8°C. Do not freeze.	Stable until expiry date (EXP) on label.	A suspension of magnetic particles in TBS.

BSA - bovine serum albumin; PBS - phosphate-buffered saline; TBS - TRIS-buffered saline

Components may be shipped at room temperature (15 - 25°C) but should be stored as indicated above.

Sample Preparation

BONE

1. Clean a mortar and pestle with 70% isopropanol. Remove the isopropanol from the mortar and pestle, and allow to air dry in a sterile biohazard safety cabinet for 30 minutes. Rinse mortar and pestle with sterile PBS just prior to use.
2. Wipe scissors and forceps with an alcohol wipe and air dry. Ensure they are completely dry as residual alcohol may reduce marrow cell viability.
3. After sacrificing the mouse:
   a. Wet the pelt thoroughly with 70% isopropanol, then clip and peel it back to expose the hind limbs.
   b. Using sterile sharp scissors, make a small incision parallel and as close to the spine as possible. Hold the foot and pull the leg towards the head to remove the entire intact leg (iliac crest, femur, and tibia) from the animal. Repeat for remaining leg.
   c. Using a scalpel, cut through the tibia just above the ankle to remove the foot. Position the leg such that the iliac crest is facing up, and holding the femur near the knee, scrape the scalpel down the femur towards the iliac crest to dislocate the hip joint. The femoral head should be visible once it has dislocated from the hip.
   d. Cut remaining muscle to remove iliac crest. Cut the knee joint in the center and remove ligaments and excess tissue.
4. Using a scalpel, scrape bones thoroughly to remove muscle and cut to remove epiphyses. Ensure that the bones are cleaned thoroughly with no remaining muscle tissue attached.
5. Place cleaned bones in the mortar containing 10 mL PBS containing 2% fetal bovine serum (FBS) and 1 mM EDTA. The solution of PBS containing 2% FBS and 1 mM EDTA is now referred to as “Buffer”.
6. Crush bones gently with pestle, using only enough force to crack open the bones. Agitate gently to free bone marrow (BM) from bone fragments and pipette off Buffer. Buffer containing BM can be filtered through a 70 µm cell strainer (Corning Catalog #352350) and used for other applications (e.g. culture expansion or to perform the CFU-F assay).

   NOTE: Use gentle pressure to crack open the bones. Cell viability will decrease and excess debris will be generated when bones are harshly ground.
7. Add 10 mL fresh Buffer and repeat agitation and removal of BM. Repeat wash step an additional 4 times (for a total of 6 washes) or until the majority of the BM has been removed (bone fragments turn white in color).
8. Transfer the bone fragments to a 100 mm dish. Add 2 mL of Collagenase Type I (0.25%; Catalog #07902). Ensure bones are completely covered in solution. Let sit for 3 - 5 minutes. This step softens the bone, allowing it to be chopped more easily.
9. Using a scalpel, chop the remaining bone fragments into fine pieces (1 - 2 mm fragments).

   NOTE: Proper bone fragmentation is required to release sufficient amounts of cells for cell separation.
10. Transfer the bone fragments and collagenase solution to a 50 mL polypropylene tube and add more Collagenase Type I (0.25%) to a final volume of 2 mL per mouse used, or a minimum of 10 mL.
11. Seal lid with Parafilm® and place tube in a shaking 37°C water bath at maximum speed for 45 minutes. If using a bacterial culture shaker, set speed to ~200 rpm.
12. After 45 minutes, remove the tube from the shaker and add Buffer to a final volume of 30 mL. Collect supernatant and filter through a 70 µm cell strainer. Wash bone fragments by mixing with an additional 10 mL of Buffer and allowing fragments to settle for 3 - 4 minutes. Filter the wash through the 70 µm strainer, combining with the previously collected cells (for a final volume of 40 mL).
13. Centrifuge at 300 x g for 10 minutes at room temperature (15 - 25°C) with the brake on and carefully remove the supernatant.
14. Resuspend the cell pellet at 2 - 5 x 10^7 cells/mL in recommended medium (small particles and debris may be visible in the cell suspension) and keep on ice until ready for use.

    NOTE: Expected cell recovery is 1.5 - 3.5 x 10^6 cells per mouse (2 femurs and 2 tibias). If the cell yield is > 5 x 10^6 cells/mouse, this is an indication that the marrow was not depleted sufficiently.

Recommended Medium

EasySep™ Buffer (Catalog #20144), or PBS containing 2% FBS and 1 mM EDTA. HBSS, Modified (Without Ca++ and Mg++; Catalog #37250) can be used in place of PBS. Medium should be free of Ca++ and Mg++.

Directions for Use – Manual EasySep™ Protocols

See page 1 for Sample Preparation and Recommended Medium. Refer to Table 1 for detailed instructions regarding the EasySep™ procedure.

Table 1. EasySep™ Mouse Mesenchymal Stem/Progenitor Cell Enrichment Kit Protocol

		EASYSEP™ MAGNET
STEP	INSTRUCTIONS	 **EasySep™ (Catalog #18000)**
1	**Prepare sample at the indicated cell concentration within the volume range.**	2 - 5 x 10^7 cells/mL 0.2 - 0.5 mL NOTE: If starting with fewer than 4 x 10^6 cells, resuspend cells in 0.2 mL
	Add sample to required tube.	5 mL (12 x 75 mm) polystyrene round-bottom tube (e.g. Catalog #38007)
2	**Add Enrichment Cocktail to sample.**	50 µL/mL of sample
	Mix and incubate.	2 - 8°C for 15 minutes
3	**Add recommended medium to sample.**	Top up to 4.5 mL
	Centrifuge.	400 x g for 5 minutes
4	**Discard supernatant and resuspend cells in recommended medium.**	Use same volume as step 1
5	**Add Selection Cocktail to sample.**	250 µL/mL of sample
	Mix and incubate.	2 - 8°C for 15 minutes
6	**Vortex Magnetic Particles.** **NOTE: Particles should appear evenly dispersed.**	30 seconds
7	**Add Magnetic Particles to sample.**	150 µL/mL of sample
	Mix and incubate.	2 - 8°C for 15 minutes
8	**Add recommended medium to top up the sample to the indicated volume. Mix by gently pipetting up and down 2 - 3 times.**	Top up to 2.5 mL
	Place the tube (without lid) into the magnet and incubate.	RT for 5 minutes
9	**Pick up the magnet, and in one continuous motion invert the magnet and tube,* pouring the enriched cell suspension into a new tube.**	Isolated cells are ready for use

RT - room temperature (15 - 25°C)

* Leave the magnet and tube inverted for 2 - 3 seconds, then return upright. Do not shake or blot off any drops that may remain hanging from the mouth of the tube.

Notes and Tips

EXPANSION PROTOCOL

Harvest and process mouse compact bone cells as described in Sample Preparation, page 2.

SAMPLE	START COMPACT BONE		ENRICHED COMPACT BONE	
CULTURE CONDITION	HYPOXIC ENVIRONMENT*		HYPOXIC ENVIRONMENT*	
Volume and medium to use	2 mL Complete MesenCult™ Medium (Mouse)	10 mL Complete MesenCult™ Medium (Mouse)	2 mL Complete MesenCult™ Medium (Mouse)	10 mL Complete MesenCult™ Medium (Mouse)
Cell number to plate	2 - 5 x 10^5 cells/well in a 6-well plate	0.6 - 1.2 x 10^6 cells in a T-25 cm^2 tissue culture flask	1 - 5 x 10^4 cells/well in a 6-well plate	0.4 - 1.2 x 10^5 cells in a T-25 cm^2 tissue culture flask

* Hypoxic environment refers to 5% O_2, 10% CO_2. If culture in a normal environment (20% O_2, 5% CO_2) is preferred, plate 5 - 10X more cells and optimize for your own experimental conditions.

1. Incubate cells at 37°C in a hypoxic environment using a Hypoxia Incubator Chamber (Catalog #27310) for 8 - 14 days or a normal environment for 10 - 21 days until an adherent cell layer has formed. After 8 days, if the color of the medium has become orange, a half-medium change can be performed.
2. Observe mesenchymal cells under the microscope after 7 days to determine confluency. Once the cells have reached 80% confluency, they are ready to be passaged; 80% confluency may be reached in 7 - 16 days.
3. Passage according to the protocol provided in the Product Information Sheet for MesenCult™ (Mouse; Document #29566).

ASSESSING PURITY

The depletion of non-mesenchymal progenitor cells by flow cytometry can be assessed using the following fluorochrome-conjugated antibody clones:

- Anti-Mouse CD45 Antibody, Clone 30-F11 (Catalog #60030), and
- Anti-Mouse TER119 Antibody, Clone TER-119 (Catalog #60033)

The following method may also be used to assess purity:

Mouse colony forming unit – fibroblast (CFU-F) assays initiated from compact bone cells should be initiated at 3 different cell concentrations to ensure that there are sufficient colony numbers to accurately assess the progenitor cell content of the mouse bone cells.

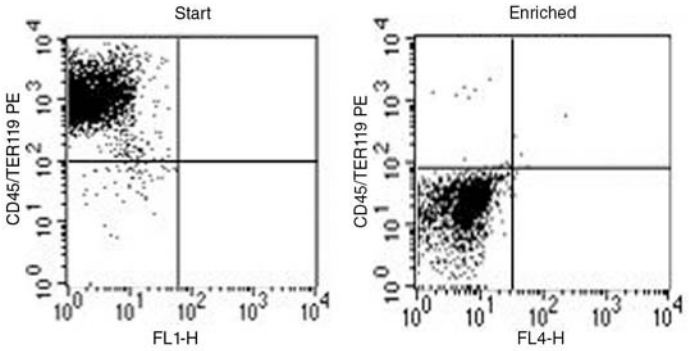
SAMPLE	START COMPACT BONE		ENRICHED COMPACT BONE	
CULTURE CONDITION	HYPOXIC ENVIRONMENT*	NORMAL ENVIRONMENT	HYPOXIC ENVIRONMENT*	NORMAL ENVIRONMENT
Volume and medium to use	2 mL Complete MesenCult™ Medium (Mouse)	2 mL Complete MesenCult™ Medium (Mouse)	2 mL Complete MesenCult™ Medium (Mouse)	2 mL Complete MesenCult™ Medium (Mouse)
Cell number to plate	1,000, 5,000 and 10,000 cells/cm^2 in duplicate in a 6-well plate (e.g. plate 10,000, 50,000 and 100,000 cells/well)	10,000, 20,000, and 40,000 cells/cm^2 in duplicate in a 6-well plate (e.g. plate 100,000, 200,000 and 400,000 cells/well)	50 - 250 cells/cm^2 in duplicate in a 6-well plate (e.g. plate 500 - 2,500 cells/well)	200 - 1000 cells/cm^2 in duplicate in a 6-well plate (e.g. plate 2000 - 10,000 cells/well)

* Hypoxic environment refers to 5% O_2, 10% CO_2; Normal environment refers to 20% O_2, 5% CO_2

Incubate cells at 37°C in desired atmosphere for 10 - 13 days. No medium change is required.

Cells from compact bone grown in the CFU-F assay occasionally undergo spontaneous differentiation to form adipocytic or chondrogenic cells. For additional information, contact us at techsupport@stemcell.com.

Data



Starting with mouse compact bone cells, the CD45-TER119- cell content of the enriched fraction typically ranges from 50 - 99%. In the above example, the purities of the start and final enriched fractions are 1.4% and 99.1%, respectively. CFU-F enrichment is typically 50 - 200 fold.