

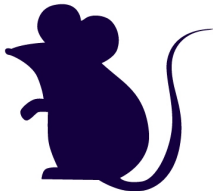
PROCEDURE **Negative Selection**

+EasySep[®]

Mouse Mesenchymal Progenitor Enrichment Kit for Compact Bone

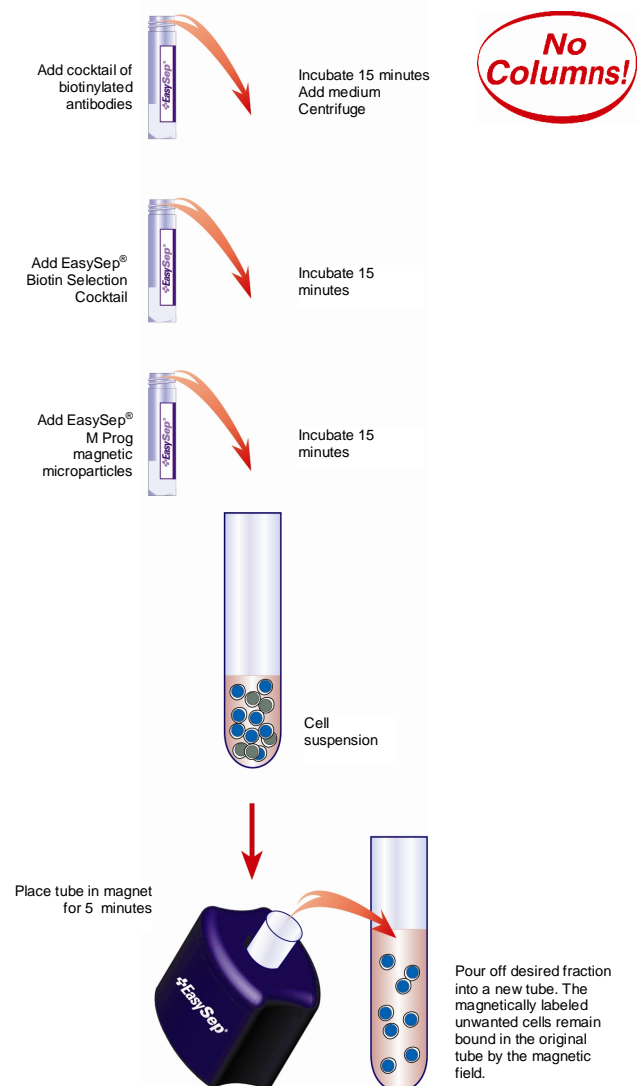
Version 1.0.0

CATALOG #19771



This Product Information Sheet is provided for use with the Purple EasySep[®] magnet. This product is NOT compatible with "The Big Easy" Silver EasySep[®] magnet or with RoboSep[®].

Manual EasySep[®] Protocol Diagram



Manual EasySep[®] Protocol Using Purple EasySep[®] Magnet (Catalog #18000).

This procedure is used for processing 200 - 500 μL of sample (up to 2.5×10^7 cells)

1. Prepare single nucleated cell suspension at a concentration of $2 - 5 \times 10^7$ cells/mL in recommended medium (see Notes and Tips, reverse side). For samples containing 4×10^6 cells or fewer, resuspend in 200 μL . Cells must be placed in a 5 mL (12 x 75 mm) polystyrene tube to properly fit into the purple EasySep[®] Magnet.

Falcon[™] 5 mL (BD Catalog #352058) Polystyrene Round-Bottom Tubes are recommended.

2. Add Mesenchymal Progenitor Cell Enrichment Cocktail containing biotinylated antibodies at **50 $\mu\text{L}/\text{mL}$ of cells** (e.g. for 0.5 mL of cells, add 25 μL of cocktail). Mix well and incubate at 2 - 8 °C for **15 minutes**.
3. Add 4 mL of recommended medium and centrifuge cells for 5 minutes at 400 x g. Resuspend cells at $2 - 5 \times 10^7$ cells/mL in recommended medium.
4. Add Biotin Selection Cocktail at **250 $\mu\text{L}/\text{mL}$ of cells** (e.g. for 0.5 mL of cells, add 125 μL of selection cocktail). Mix well and incubate at 2 - 8 °C for **15 minutes**.
5. Vortex EasySep[®] Mouse Progenitor (M Prog) Magnetic Microparticles for 30 seconds to ensure that the particles are in a uniform suspension with no visible aggregates.
6. Add the microparticles at **250 $\mu\text{L}/\text{mL}$ of cells** (e.g. for 0.5 mL of cells, add 125 μL of microparticles). Mix well and incubate at 2 - 8 °C for **15 minutes**.
7. Bring the cell suspension to a **total volume** of 2.5 mL by adding recommended medium. Mix the cells in the tube by pipetting gently 2 - 3 times. Place the tube (without cap) into the magnet. Set aside for **5 minutes**.
8. Pick up the EasySep[®] Magnet, and in one continuous motion invert the magnet and tube, pouring off the desired fraction into a new 5 mL polystyrene tube. The magnetically labeled unwanted cells will remain bound inside the original tube, held by the magnetic field of the magnet. Leave the magnet and the tube inverted for 2 - 3 seconds, then return to upright position. **Do not shake or blot off any drops that may remain hanging from the mouth of the tube.** The enriched cells in the new tube are now ready for use.

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November 2007

FOR RESEARCH USE ONLY

#29122

Catalog #19771For labeling up to 4×10^8 total cells

Components:

- EasySep[®] Negative Selection Mouse Mesenchymal Progenitor Enrichment Cocktail 0.4 mL
- EasySep[®] Biotin Selection Cocktail 2 x 1.0 mL
- EasySep[®] M Prog Magnetic Microparticles 2 x 1.0 mL

REQUIRED EQUIPMENT:

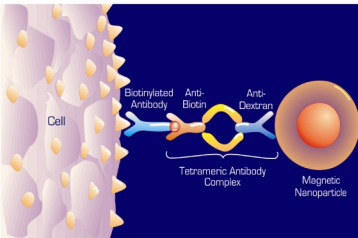
EasySep[®] Magnet (Catalog #18000). This product is not compatible with "The Big Easy" EasySep[®] Magnet or RoboSep[®].

PRODUCT DESCRIPTION AND APPLICATIONS:

EasySep[®] Negative Selection Mouse Mesenchymal Progenitor Enrichment Cocktail, EasySep[®] Biotin Selection Cocktail and EasySep[®] M Prog Magnetic Microparticles label non-mesenchymal progenitor cells from mouse compact bone for magnetic separation. These reagents are designed to enrich mesenchymal stem cells and progenitor cells from mouse bone cell suspensions by depletion of non-mesenchymal progenitor cells.

EASYSEP[®] LABELING OF MOUSE CELLS:

Unwanted cells are specifically labeled with dextran-coated magnetic microparticles using biotinylated antibodies against cell surface antigens expressed on the unwanted cells, and bispecific Tetrameric Antibody Complexes (TAC). These complexes recognize both dextran and biotin (Figure 1). The small size of the magnetic dextran iron particles allows for efficient binding to the TAC-labeled cells. Magnetically labeled cells are then separated from unlabeled target cells using the EasySep[®] procedure (reverse side).

**Figure 1.**

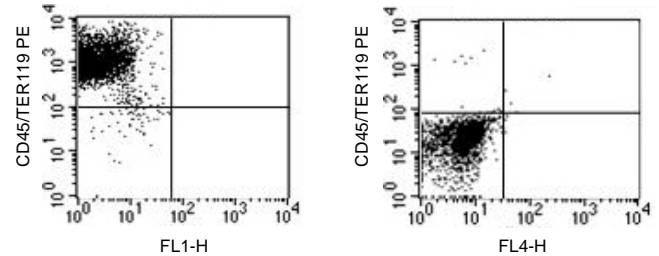
Schematic Drawing of EasySep[®] TAC Magnetic Labeling of Mouse Cells.

NOTES AND TIPS:**Preparing a Single Cell Suspension.**

Bone: Collect cells from the tibia and femur by crushing the bones, according to the method described in the Compact Bone Preparation Procedure for EasySep[®] Mouse Mesenchymal Progenitor Enrichment (Catalog #29134, provided). Resuspend cells at a concentration of $2 - 5 \times 10^7$ cells/mL in recommended medium. The final volume should be between 200 and 500 μ L. Expect $1.5 - 3.5 \times 10^5$ cells per mouse.

Recommended Medium. For the EasySep[®] procedure, the recommended medium is Phosphate Buffered Saline (PBS) + 2% Fetal Bovine Serum (FBS) (Catalog #07905), and 1 mM EDTA. Medium should be Ca^{++} and Mg^{++} free. Hank's Balanced Salt Solution can be used in place of PBS. When assessing purity using the CFU-F Assay, cells should be resuspended in Complete MesenCult[®] Medium (Mouse) (Catalog #05511).

Assessing Purity. The depletion of non-mesenchymal progenitor cells can be assessed by flow cytometry after staining with fluorochrome-conjugated antibodies against CD45 (e.g. PE anti-CD45, Catalog #10809) and TER119 (e.g. PE anti-TER119, Catalog #10821). Purity can also be assessed using the CFU-F Assay as described in the Compact Bone Preparation Procedure (Catalog #29134, provided).

TYPICAL EASYSEP[®] MOUSE MESENCHYMAL PROGENITOR CELL ENRICHMENT PROFILE:

Start: 1.4% CD45⁺TER119⁺ Cells Enriched: 99.1% CD45⁺TER119⁺ Cells
The CD45⁺TER119⁺ cell content of the enriched cells typically ranges from 50 - 99%.
CFU-F enrichment: 50 - 200 fold

COMPONENT DESCRIPTIONS:**EasySep[®] Negative Selection Mouse****code #19771C****Mesenchymal Progenitor Enrichment Cocktail**

This cocktail contains a combination of biotinylated monoclonal antibodies purified from rat ascites fluid or hybridoma culture supernatant. The monoclonal antibodies are purified by affinity chromatography using Protein A or Protein G Sepharose. These antibodies are directed against cell surface antigens on mouse cells of hematopoietic origin (CD45, TER119). This cocktail is supplied in PBS. It should be noted that this product is a biological reagent, and as such cannot be completely characterized or quantified. Some variability is unavoidable.

EasySep[®] Biotin Selection Cocktail**code #18153**

This cocktail is a combination of two mouse IgG₁ monoclonal antibodies directed against biotin and dextran, purified from hybridoma culture supernatant. These antibodies are bound in bispecific Tetrameric Antibody Complexes by rat monoclonal antibodies directed against mouse IgG₁. This cocktail is supplied in PBS. It should be noted that this product is a biological reagent, and as such cannot be completely characterized or quantified. Some variability is unavoidable.

EasySep[®] Mouse Progenitor (M Prog)**code #19350****Magnetic Microparticles**

A suspension of magnetic dextran iron particles in TRIS buffer.

STABILITY AND STORAGE:**EasySep[®] Negative Selection Mouse Mesenchymal Progenitor Enrichment Cocktail**

Stable at 4°C for 1 year. Do not freeze this product. Contents sterile in unopened tube. This product may be shipped at room temperature, and should be refrigerated upon receipt.

EasySep[®] Biotin Selection Cocktail

Stable at 4°C for 2 years. Do not freeze this product. Contents sterile in unopened tube. This product may be shipped at room temperature, and should be refrigerated upon receipt.

EasySep[®] Mouse Progenitor (M Prog) Magnetic Microparticles

Stable at 4°C for 1 year. Contents sterile in unopened tube. This product may be shipped at room temperature, and should be refrigerated upon receipt.

New Product - 25% extra free in exchange for your feedback!

You may have noticed that this first batch of the new EasySep[®] Mouse Mesenchymal Progenitor Enrichment Kit for Compact Bone is provided with a 25% excess of the Mesenchymal Progenitor Enrichment Cocktail (Catalog #19771C). If you would like to receive additional Biotin Selection Cocktail and Magnetic Microparticles in order to take advantage of this extra reagent, please contact StemCell Technologies Technical Support at techsupport@stemcell.com with your feedback on this new product. We will then send you the additional reagents to thank you for your time.

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