PROCEDURE

EasySep™

NEGATIVE SELECTION

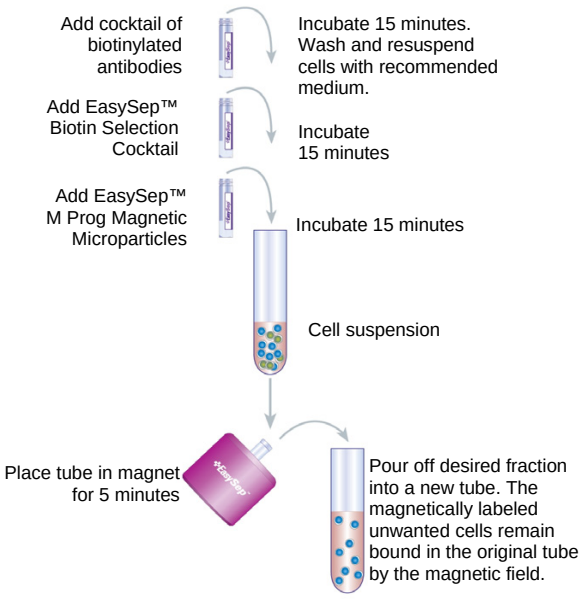
# MOUSE MESENCHYMAL PROGENITOR ENRICHMENT KIT

FOR COMPACT BONE

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 x $10^7$ cells).

1. Prepare cell suspension at a concentration of 2 - 5 x $10^7$ cells/mL in recommended medium (see Notes and Tips, reverse side). For samples containing 4 x $10^6$ 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 Polystyrene Round-Bottom Tubes (BD Biosciences, Catalog #352058) are recommended.*

2. Add the EasySep™ Mouse Mesenchymal Progenitor Enrichment Cocktail at **50 µL/mL of cells** (e.g. for 0.5 mL of cells, add 25 µL of cocktail). Mix well and incubate in refrigerator (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 x $10^7$ cells/mL in recommended medium.

4. Add the EasySep™ Biotin Selection cocktail at **250 µL/mL of cells** (e.g. for 0.5 mL of cells, add 125 µL of selection cocktail). Mix well and incubate in refrigerator (2 - 8°C) for **15 minutes**.

5. Vortex the EasySep™ M Prog Magnetic Microparticles for 30 seconds. Ensure that the particles are in a uniform suspension with no visible aggregates.

6. Add the EasySep™ M Prog Magnetic Microparticles at **150 µL/mL of cells** (e.g. for 2 mL of cells, add 300 µL of magnetic particles). Mix well and incubate in refrigerator (2 - 8°C) for **15 minutes**.

7. Bring the cell suspension up to a **total volume** of **2.5 mL** by adding recommended medium. Mix the cells in the tube by gently pipetting up and down 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 EasySep™ Magnet. Leave the magnet and tube in inverted position 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 negatively selected, enriched cells in the new tube are now ready for use.

CATALOG #19771 For labeling 4 x $10^8$ 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

EasySep™ NEGATIVE SELECTION

## REQUIRED EQUIPMENT:

EasySep™ Magnet (Catalog #18000). This product is not compatible with "The BigEasy" 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 particles using biotinylated antibodies against cell surface antigens expressed on the unwanted cells, and bispecific Tetrameric Antibody Complexes (TACs). These complexes recognize both dextran and biotin (Figure 1). Magnetically labeled cells are then separated from unlabeled target cells using the EasySep™ procedure (reverse side).

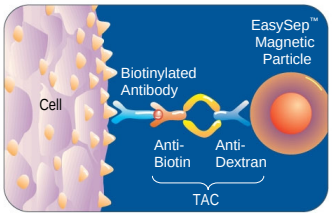



**Figure 1.**
Schematic Drawing of EasySep™ TAC Magnetic Labeling of Mouse Cells.

## NOTES AND TIPS:

### PREPARING THE CELL SUSPENSION.

**BONE**

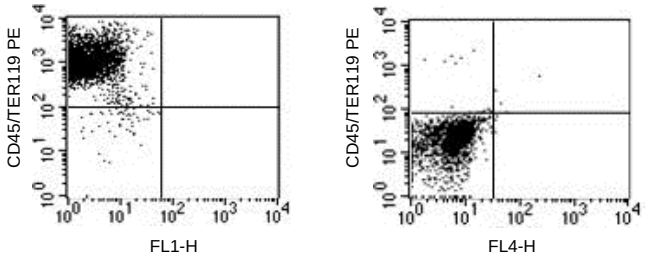
Collect cells from the tibia and femur by crushing the bones, according to the method described in the Compact Bone Preparation Procedure for EasySep™ Mouse Mesenchymal Progenitor Enrichment (Catalog #29134, provided). Resuspend cells at a concentration of 2 - 5 x $10^7$ cells/mL in recommended medium. The final volume should be between 200 and 500 µL. Expect 1.5 - 3.5 x $10^6$ cells per mouse.

**RECOMMENDED MEDIUM.** The recommended medium is phosphate-buffered saline (PBS) + 2% fetal bovine serum (FBS) (Catalog #07905) with 1 mM EDTA added. 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 MESENCHYMAL PROGENITOR ENRICHMENT COCKTAIL** **CODE #19771C**

This cocktail contains a combination of biotinylated monoclonal antibodies. These 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 bound in bispecific TACs by rat monoclonal antibodies 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) MAGNETIC MICROPARTICLES** **CODE #19350**

A suspension of magnetic dextran iron particles in TRIS buffer.

## STABILITY AND STORAGE:

**EASYSEP™ NEGATIVE SELECTION MOUSE MESENCHYMAL PROGENITOR ENRICHMENT COCKTAIL**

**EASYSEP™ BIOTIN SELECTION COCKTAIL**

**EASYSEP™ MOUSE PROGENITOR (M PROG) MAGNETIC MICROPARTICLES**

Product stable at 2 - 8°C until expiry date as indicated on label. Contents have been sterility tested. Do not freeze this product. This product may be shipped at room temperature (15 - 25°C), and should be refrigerated upon receipt.



STEMCELL™ TECHNOLOGIES
TOLL-FREE T. 1 800 667 0322 • T. +1 604 877 0713 • TOLL-FREE F. 1 800 567 2899 • F. +1 604 877 0704
ORDERS@STEMCELL.COM • INFO@STEMCELL.COM • FOR FULL CONTACT DETAILS WORLDWIDE VISIT WWW.STEMCELL.COM