PROCEDURE

EasySep™

NEGATIVE SELECTION

# MOUSE EPITHELIAL CELL ENRICHMENT KIT

CATALOG #19758

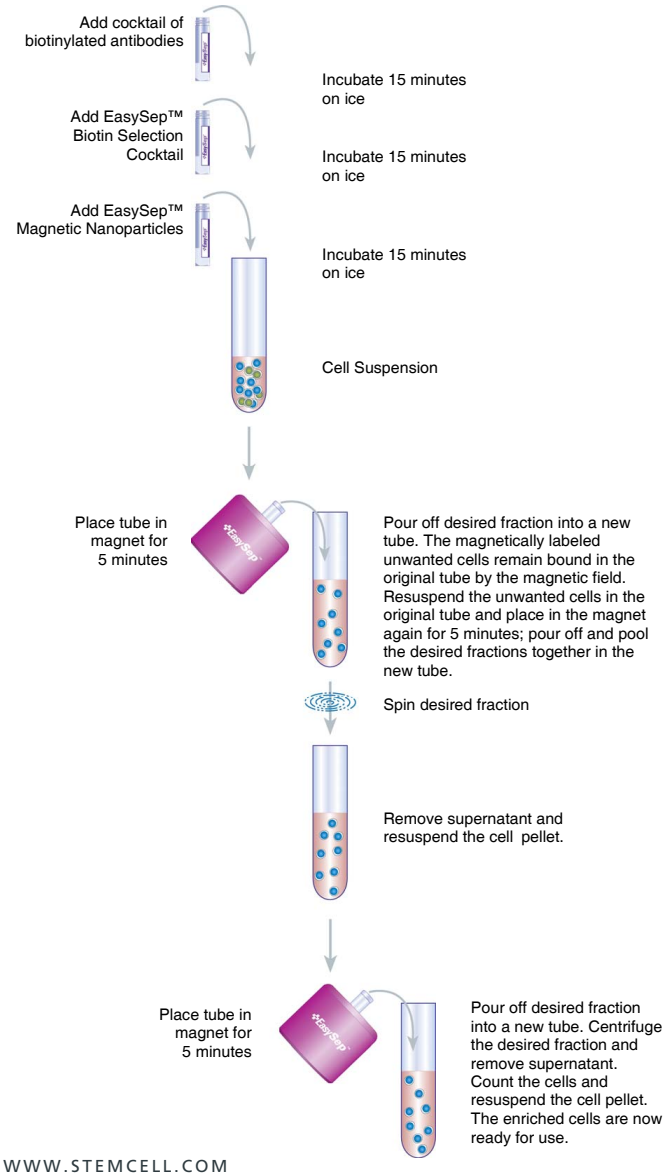
STEMCELL TECHNOLOGIES

V 1.1.0

**THIS PRODUCT INFORMATION SHEET IS PROVIDED FOR USE WITH THE PURPLE EASYSEP™ MAGNET (18000) ONLY.**

**IMPORTANT NOTE:** This procedure is designed for use with the Purple EasySep™ Magnet (18000). If using "The Big Easy" EasySep™ Magnet (18001), please refer to the Product Information Sheet for this magnet (Catalog #28897). For more information, please contact our Technical Support group at techsupport@stemcell.com.

## MANUAL EASYSEP™ PROTOCOL DIAGRAM




## A) MANUAL EASYSEP™ PROTOCOL USING THE PURPLE EASYSEP™ MAGNET (CATALOG #18000).

1. Prepare single cell suspension at a concentration of 1 x $10^8$ cells/mL in recommended medium (see Notes and Tips, reverse side) supplemented with 0.1 mg/mL DNase I (Catalog #07900). Cells must be placed in a 5 mL (12 x 75 mm) polystyrene tube to properly fit into the Purple EasySep™ Magnet.

   *Note: Do not exceed a volume of 2.0 mL (i.e. 2 x $10^8$ cells) per tube. If starting with fewer than 2 x $10^7$ cells, resuspend the cells in a minimum volume of 200 µL of recommended medium.*

   *Falcon™ 5 mL Polystyrene Round-Bottom Tubes (BD Biosciences, Catalog #352058) are recommended.*

2. Centrifuge the tube of EasySep™ Mouse Epithelial Cell Enrichment Cocktail before use to ensure recovery of entire contents. Add the cocktail at **50 µL/mL cells** (e.g. for 2 mL of cells, add 100 µL of cocktail). Mix well and incubate on ice for **15 minutes**.

3. Add the EasySep™ Biotin Selection Cocktail at **100 µL/mL cells** (e.g. for 2 mL of cells, add 200 µL of selection cocktail). Mix well and incubate on ice for **15 minutes**.

4. Mix the EasySep™ Magnetic Nanoparticles to ensure that particles are in a uniform suspension by vigorously pipetting more than 5 times. *Vortexing is not recommended.* Add the nanoparticles at **50 µL/ mL cells** (e.g. for 2 mL of cells, add 100 µL of nanoparticles). Mix well and incubate on ice for **15 minutes**.

   *Optional: Take a small aliquot of cell suspension to assess purity (see Notes and Tips, reverse side)*

5. Bring the cell suspension 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**.

6. 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 (12 x 75 mm) 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.*

7. To maximize cell recovery, remove the original tube containing the magnetically labeled unwanted cells from the EasySep™ Magnet and add another 2.0 mL of recommended medium to this tube. 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 the 5 mL (12 x 75 mm) polystyrene tube that contains the desired cells from the first separation. This tube should now have a **total volume** of approximately **4.5 mL**.

9. Centrifuge this suspension of desired cells at 350 x *g* for **5 minutes**.

10. Discard the supernatant and resuspend the cell pellet to a **total volume** of **2.5 mL** in recommended medium. Place the tube (without cap) into the magnet. Set aside for **5 minutes**.

11. 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 (12 x 75 mm) 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 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.*

12. Centrifuge the poured-off cell suspension at 350 x *g* for **5 minutes**. Discard the supernatant. Count the cells and resuspend at any desired cell concentration in recommended medium. The enriched cells are now ready for use.

    *Optional: Take a small aliquot of cell suspension to assess purity (see Notes and Tips, reverse side)*

CATALOG #19758 For labeling $10^9$ total cells

Components:

- EasySep™ Mouse Epithelial Cell Enrichment Cocktail 0.5 mL
- EasySep™ Biotin Selection Cocktail 1.0 mL
- EasySep™ Magnetic Nanoparticles 1.0 mL

EasySep™ NEGATIVE SELECTION

## REQUIRED EQUIPMENT:

Purple EasySep™ Magnet (Catalog #18000). or "The Big Easy" EasySep™ Magnet (Catalog #18001).

## PRODUCT DESCRIPTION AND APPLICATIONS:

EasySep™ Mouse Epithelial Cell Enrichment Cocktail, EasySep™ Biotin Selection Cocktail and EasySep™ Magnetic Nanoparticles label non-epithelial cells for magnetic separation. These reagents are designed to enrich epithelial cells from mouse cell suspensions by depletion of hematopoietic, endothelial, and some fibroblast cells.

## EASYSEP™ LABELING OF MOUSE CELLS:

Unwanted cells are specifically labeled with dextran-coated magnetic nanoparticles 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).

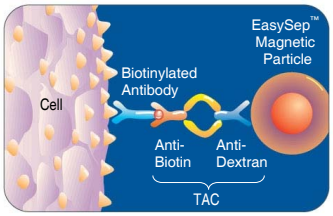



**Figure 1.**
Schematic Drawing of EasySep™ TAC Magnetic Labeling of Mouse Cells.

## NOTES AND TIPS:

**PREPARING A MONONUCLEAR CELL SUSPENSION.** Collagenase/Hyalouronidase Solution (Catalog #07912) or Gentle Collagenase/Hyaluronidase (Catalog #07919) are recommended. Please refer to these products' Product Information Sheets (Catalog #29634 and 29629) for detailed information on the recommended protocol. For more information on how to dissociate solid tissues, please visit www.stemcell.com and download our Technical Bulletin - A Guide To Solid Tissue Dissociation (Catalog #29107).

**RECOMMENDED MEDIUM.** The recommended medium is Hanks' Balanced Salt Solution (HBSS) with 10mM HEPES, Without Phenol Red (Catalog #37150) + 2% Fetal Bovine Serum.

**MINIMIZING CELL CLUMPING.** To prevent clumping, always use cold buffers and keep cells on ice as much as possible. If sample begins to clump, add 1 mg/mL DNase I (Catalog #07900) and gently pipette cells to disaggregate.

**RECOMMENDATIONS.** For further selection of mouse mammary stem cells, we recommend the EasySep™ Mouse Mammary Stem Cell Enrichment Kit (Catalog #19757) which contains two additional antibodies for the selection of mammary epithelial stem cells. In addition, we also recommend the EpiCult™- B Mouse Medium Kit (Catalog #05610) as a medium for the growth and culture of mouse mammary progenitors.

**ASSESSING PURITY.** Purity of epithelial cells can be measured by flow cytometry after staining with a fluorochrome-conjugated secondary antibody such as FITC-labeled goat anti-rat IgG (Jackson ImmunoResearch, Catalog #112-095-167). FITC-labeled goat anti-rat IgG detects the primary antibodies used to deplete mouse cells of hematopoietic and endothelial origin (cells expressing CD45, Ter119 and/or CD31).

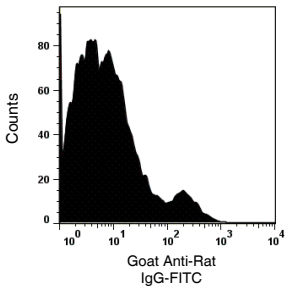
To assess the depletion of mouse hematopoietic and endothelial cells, take a small aliquot of non-enriched cells at the end of Step 4 (page 1) and wash cells once by topping up the sample tube with recommended media and centrifuge at 350 x *g* for 5 minutes. Discard the supernatant and resuspend the cells in recommended media. Take a small aliquot of enriched cells at the end of cell separation procedure (Step 12, page 1) and stain both non-enriched and enriched cells with the secondary antibody according to manufacturer's recommendations. Assess purity by flow cytometry.

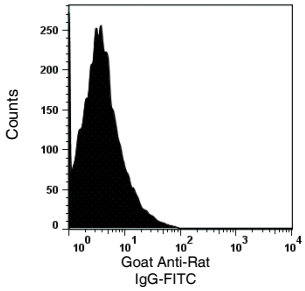
## TYPICAL EASYSEP™ DEPLETION PROFILE:

Start: 10.37 ± 0.50% of cells expressing one or more of the following markers: CD45, Ter119, CD31, and/or BP-1

Enriched: 1.12 ± 0.22% of cells expressing one or more of the following markers: CD45, Ter119, CD31, and/or BP-1

The percentage of hematopoietic, endothelial, and fibroblast cells after separation is 1.12 ± 0.22%.




Purity has been assessed by staining with goat anti-rat IgG-FITC, which recognizes the antibodies used to deplete cells expressing CD45, Ter119, CD31, and/or BP-1.

## COMPONENT DESCRIPTIONS:

**EASYSEP™ MOUSE EPITHELIAL CELL ENRICHMENT COCKTAIL** **CODE #19757C.1**

This cocktail contains a combination of biotinylated monoclonal antibodies which are directed against cell surface antigens on mouse cells of hematopoietic, endothelial, and fibroblast origin (CD45, TER119, CD31 and BP-1). 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 #19153**

This cocktail is a combination of two mouse $IgG_1$ monoclonal antibodies against biotin and dextran. These antibodies are bound in bispecific Tetrameric Antibody Complexes by rat monoclonal antibodies against mouse $IgG_1$. 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™ MAGNETIC NANOPARTICLES** **CODE #19150**

A suspension of magnetic dextran iron particles in water.

## STABILITY AND STORAGE:

**EASYSEP™ MOUSE EPITHELIAL CELL ENRICHMENT COCKTAIL**

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.

**EASYSEP™ BIOTIN SELECTION COCKTAIL**

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.

**EASYSEP™ MAGNETIC NANOPARTICLES**

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.



IN NORTH AMERICA TOLL-FREE T. 1 800 667 0322 TOLL-FREE F. 1 800 567 2899 T. +1 604 877 0713 F. +1 604 877 0704 E. INFO@STEMCELL.COM
IN EUROPE TOLL-FREE T. 00 800 7836 2355 TOLL-FREE F. 00 800 7836 2300 E. INFO.EU@STEMCELL.COM
IN AUSTRALIA TOLL-FREE T. 1 800 060 350 F. +61 (03) 9338 4320 E. INFO.AUS@STEMCELL.COM
IN SINGAPORE T. +(65) 6776 7754 F. +(65) 6776 7114 E. INFO.SG@STEMCELL.COM


V1.1.0
#29165