PROCEDURE

EasySep®

NEGATIVE SELECTION

MOUSE NK CELL ENRICHMENT KIT

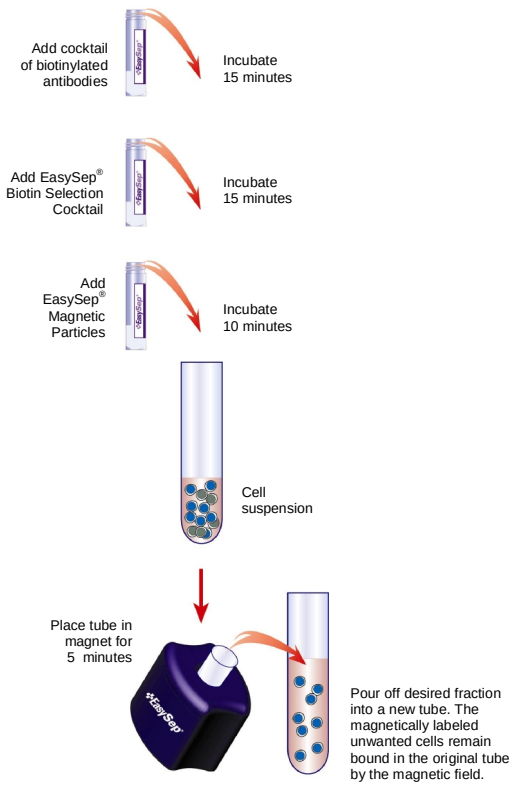
CATALOG #19755

STEMCELL TECHNOLOGIES

V 3.1.0

THIS PRODUCT INFORMATION SHEET IS PROVIDED FOR USE WITH THE PURPLE EASYSEP® MAGNET (SECTION A) OR "THE BIG EASY" SILVER EASYSEP® MAGNET (SECTION B). THIS PRODUCT IS NOT COMPATIBLE WITH ROBOSEP® - THE FULLY AUTOMATED CELL SEPARATOR.

MANUAL EASYSEP® PROTOCOL DIAGRAM



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

This procedure is used for processing **500 µL - 1.5 mL** of sample (up to 1.5 x 10^8 cells). For optimal performance, all steps should be performed at room temperature (15 - 25°C) (see Notes and Tips, reverse side).

1. Prepare nucleated single cell suspension at a concentration of 1 x 10^8 cells/mL in recommended medium (see Notes and Tips). Cells must be placed in a 5 mL (12 x 75 mm) polystyrene tube to properly fit into the EasySep® Magnet.

   Falcon™ 5 mL Polystyrene Round-Bottom Tubes (BD, Catalog #352058) are recommended.
2. Centrifuge the tube of EasySep® Negative Selection Mouse NK Cell Enrichment Cocktail before use to ensure recovery of entire contents. Add cocktail at **50 µL/mL of cells** (e.g. for 1 mL of cells, add 50 µL of cocktail). Mix well and incubate at room temperature (15 - 25°C) for **15** minutes.
3. Add EasySep® Biotin Selection Cocktail at **200 µL/mL cells** (e.g. for 1 mL of cells, add 200 µL of selection cocktail). Mix well and incubate at room temperature (15 - 25°C) for **15** minutes.
4. Vortex the EasySep® D Magnetic Particles for 30 seconds. Ensure that the particles are in uniform suspension with no visible aggregates.
5. Add the magnetic particles at **200 µL/mL cells** (e.g. for 1 mL of cells, add 200 µL of magnetic particles). Mix well and incubate at room temperature (15 - 25°C) for **10** minutes.
6. 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.
7. Pick up the EasySep® Magnet, and in one continuous motion invert the magnet and tube, pouring off the desired fraction into a new 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.*
8. Remove the original tube containing the magnetically labeled unwanted cells from the EasySep® Magnet and place the new tube containing the desired enriched cells inside the magnet to perform a second round of magnetic separation. Set aside for **5** minutes and repeat Step 7. The enriched cells are now ready for use.

B) MANUAL EASYSEP® PROTOCOL USING "THE BIG EASY" SILVER EASYSEP® MAGNET (CATALOG #18001).

This procedure is used for processing **1 - 5 mL** of sample (up to 5 x 10^8 cells). For optimal performance, all steps should be performed at room temperature (15 - 25°C) (see Notes and Tips, reverse side).

1. Prepare nucleated single cell suspension at a concentration of 1 x 10^8 cells/mL in recommended medium (See Notes and Tips). Cells must be placed in a 14 mL (17 x 100 mm) polystyrene tube to properly fit into the silver magnet.

   Falcon™ 14 mL Polystyrene Round-Bottom Tubes (BD, Catalog #352057) are recommended.
2. Centrifuge the tube of EasySep® Negative Selection Mouse NK Cell Enrichment Cocktail before use to ensure recovery of entire contents. Add cocktail at **50 µL/mL of cells** (e.g. for 2 mL of cells, add 100 µL of cocktail). Mix well and incubate at room temperature (15 - 25°C) for **15** minutes.
3. Add EasySep® Biotin Selection Cocktail at **200 µL/mL cells** (e.g. for 2 mL of cells, add 400 µL of selection cocktail). Mix well and incubate at room temperature (15 - 25°C) for **15** minutes.
4. Vortex the EasySep® D Magnetic Particles for 30 seconds. Ensure that the particles are in uniform suspension with no visible aggregates.
5. Add the magnetic particles at **200 µL/mL cells** (e.g. for 2 mL of cells, add 400 µL of magnetic particles). Mix well and incubate at room temperature (15 - 25°C) for **10** minutes.
6. Bring the cell suspension to a **total volume** of 5 mL (for <2 x 10^8 cells) or 10 mL (for 2 - 5 x 10^8 cells) 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.
7. Pick up the EasySep® Magnet, and in one continuous motion invert the magnet and tube, pouring off the desired fraction into a new 14 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 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.*
8. Remove the original tube containing the magnetically labeled unwanted cells from the EasySep® Magnet and place the new tube containing the desired enriched cells inside the magnet to perform a second round of magnetic separation. Set aside for **5** minutes and repeat Step 7. The enriched cells are now ready for use.

CATALOG #19755 For labeling 10^9 total cells

Components:

- EasySep® Negative Selection Mouse NK Cell Enrichment Cocktail 0.5 mL
- EasySep® Biotin Selection Cocktail 2 x 1.0 mL
- EasySep® D Magnetic Particles 2 x 1.0 mL

EasySep® NEGATIVE SELECTION

REQUIRED EQUIPMENT:

EasySep® Magnet (Catalog #18000), or "The Big Easy" EasySep® Magnet (Catalog #18001). This product is not compatible with RoboSep® - the fully automated cell separator.

PRODUCT DESCRIPTION AND APPLICATIONS:

EasySep® Negative Selection Mouse NK Cell Enrichment Cocktail, EasySep® Biotin Selection Cocktail and EasySep® D Magnetic Particles label non-NK cells for magnetic separation. These reagents are designed to enrich NK cells from mouse spleen cell suspensions by depletion of non-NK 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 (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 (opposite page).

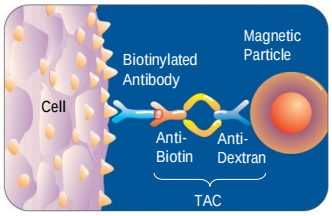


Figure 1.
Schematic Drawing of EasySep® TAC Magnetic Labeling of Mouse Cells.

NOTES AND TIPS:

PREPARING A MONONUCLEAR CELL SUSPENSION. Disrupt spleen in 5 mL of recommended medium. To remove unwanted debris and clumps, pass the cell suspension through a 70 µm nylon mesh filter into a 50 mL conical tube, and rinse with up to 20 mL of recommended medium. Centrifuge and resuspend at 1 x 10^8 nucleated cells/mL in recommended medium. Ammonium chloride treatment is not recommended when preparing the cells for separation.

OPTIMAL CELL NUMBER. We do not recommend the use of fewer than 5 x 10^7 cells per separation as this may result in sub-optimal performance.

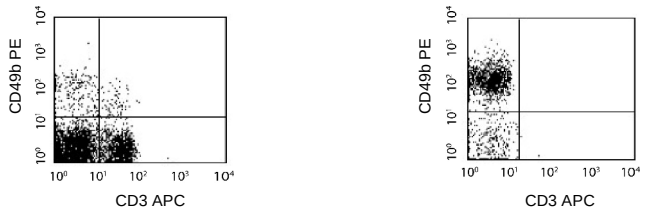
RECOMMENDED MEDIUM. The recommended medium is PBS + 2% Fetal Bovine Serum (FBS) (Catalog #07905) containing 1 mM EDTA. Medium should be Ca^{++} and Mg^{++} free.

OPTIMAL TEMPERATURE. This procedure has been optimized at room temperature (15 - 25°C). However, it can also be performed at 4°C by pre-chilling cell suspension, recommended medium and magnet, and by performing all incubation steps in a refrigerator. Under these conditions, we strongly recommend that 5% normal rat serum (Catalog #13551) be added to the cells prior to enrichment. The serum is used to prevent non-specific binding of rat antibodies to mouse cells. Please note that no significant improvement to product performance or cell viability was found using these conditions compared to the standard room temperature (15 - 25°C) protocol.

ASSESSING PURITY. Purity of NK cells can be measured by flow cytometry after staining with fluorochrome-conjugated antibodies against CD49b (e.g. PE anti-CD49b, Catalog #10826) and CD3 (e.g. FITC anti-CD3, Catalog #10700). NK cells are $CD49b^+CD3^-$.

TYPICAL EASYSEP® MOUSE NK CELL ENRICHMENT PROFILE:

Start: 3.7% $CD49b^+CD3^-$ Cells Enriched: 79.7% $CD49b^+CD3^-$ Cells



Starting with C57BL6 mouse splenocytes, the $CD49b^+CD3^-$ cell content of the enriched fraction typically ranges from 75 - 87%.

COMPONENT DESCRIPTIONS:

EASYSEP® NEGATIVE SELECTION MOUSE NK CELL ENRICHMENT COCKTAIL code #19755C.2

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. 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 purified from hybridoma culture supernatant. 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® D MAGNETIC PARTICLES code #19250

A suspension of magnetic dextran iron particles in TRIS buffer.

STABILITY AND STORAGE:

EASYSEP® NEGATIVE SELECTION MOUSE NK 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® D MAGNETIC PARTICLES

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.