

PROCEDURE


+EasySep®
NEGATIVE SELECTION

MOUSE
CD4⁺ T CELL
PRE - ENRICHMENT
KIT

CATALOG #19772

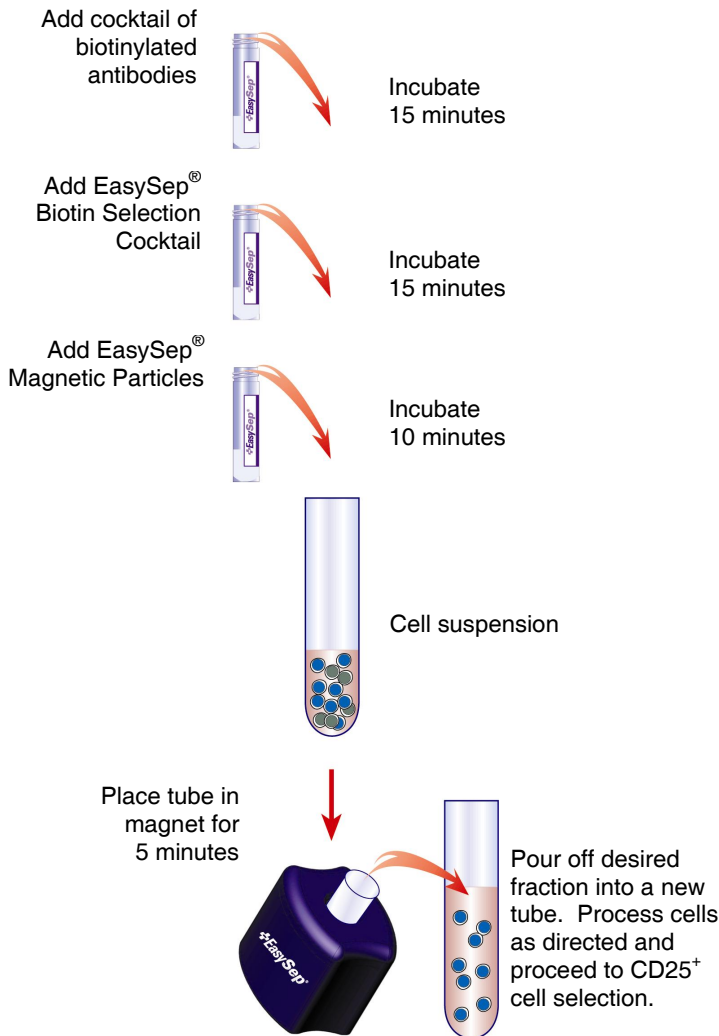
STEMCELL
TECHNOLOGIES

V 2.0.0



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 - 1.2 mL of sample ($\leq 1.8 \times 10^8$ cells).

- Prepare nucleated single cell suspension at a concentration of $1 - 1.5 \times 10^8$ cells/mL in recommended medium (see Notes and Tips, reverse side). Add 5% Normal Rat Serum (provided). Cells must be placed in a 5 mL (12 x 75 mm) polystyrene tube to properly fit into the Purple EasySep® Magnet. For samples containing fewer than 5×10^7 cells, resuspend in 500 µL.
Falcon™ 5 mL Polystyrene Round-Bottom Tubes (BD, Catalog #352058) are recommended.
- Centrifuge the tube of pre-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 - 22°C) for **15 minutes**.
- Add biotin selection cocktail at **150 µL/mL cells** (e.g. for 1 mL of cells, add 150 µL of cocktail). Mix well and incubate at room temperature (15 - 22°C) for **15 minutes**.
- Vortex the EasySep® D Magnetic Particles for 30 seconds. Ensure that the particles are in uniform suspension with no visible aggregates.
- Add the magnetic particles at **150 µL/mL cells** (e.g. for 1 mL of cells, add 150 µL of particles). Mix well and incubate at room temperature (15 - 22°C) for **10 minutes**.
- 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**.
- 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 unwanted cells will remain inside the original tube. 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.
- Add **200 µL** of EasySep® Blocking Solution (provided) to the cell suspension in the new tube. Cap tube and vortex for 3 seconds.
- Remove the original tube containing the unwanted cells from the EasySep® Magnet and place the new tube containing the desired cells inside the magnet (without cap) to perform a second round of magnetic separation. Set aside for **5 minutes** and repeat Step 7 for a total of 2 x 5-minute separations.
- Top-up the new tube to 4 mL with recommended medium and centrifuge the enriched cells at 250 x g for **10 minutes**. Remove supernatant and resuspend cells in 4 mL of recommended medium. Repeat centrifugation.
- Remove the supernatant and resuspend in 4 mL of recommended medium. Incubate at room temperature (15 - 22°C) for **20 minutes**. *Note: This 20-minute incubation is optional but may improve cell purities and/or recoveries.*
- Centrifuge at 250 x g for **10 minutes**. Resuspend the cells at a concentration of 5×10^7 cells/mL in recommended medium and proceed to CD25⁺ cell selection using the EasySep® Mouse CD25 Positive Selection Kit (Catalog #18761).
Note: If sample contains fewer than 2.5×10^7 cells, resuspend in 500 µL of medium.

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

This procedure is used for processing 1 - 5 mL of sample ($\leq 7.5 \times 10^8$ cells).

- Prepare nucleated single cell suspension at a concentration of $1 - 1.5 \times 10^8$ cells/mL in recommended medium (see Notes and Tips, reverse side). Add 5% Normal Rat Serum (provided). Cells must be placed in a 14 mL (17 x 100 mm) polystyrene tube to properly fit into the Silver EasySep® Magnet. For samples containing 1×10^8 cells or fewer, resuspend in 1 mL. *Falcon™ 14 mL Polystyrene Round-Bottom Tubes (BD, Catalog #352057) are recommended.*
- Centrifuge the tube of pre-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 - 22°C) for **15 minutes**.
- Add biotin selection cocktail at **150 µL/mL cells** (e.g. for 1 mL of cells, add 150 µL of cocktail). Mix well and incubate at room temperature (15 - 22°C) for **15 minutes**.
- Vortex EasySep® D Magnetic Particles 30 seconds to ensure that they are in uniform suspension with no visible aggregates.
- Add the magnetic particles at **150 µL/mL cells** (e.g. for 1 mL of cells, add 150 µL of particles). Mix well and incubate at room temperature (15 - 22°C) for **10 minutes**.
- Bring the cell suspension to a **total volume** of 5 mL (for < 2 mL start samples), or 10 mL (for start samples 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**.
- 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 unwanted cells will remain inside the original tube. 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.*
- Add **400 µL** of EasySep® Blocking Solution (provided) (for < 2 mL start sample) or **800 µL** (for 2 - 5 mL start sample) to the cell suspension in the new tube. Cap tube and vortex for 3 seconds.
- Remove the original tube containing the unwanted cells from the EasySep® Magnet and place the new tube containing the desired cells inside the magnet (without cap) to perform a second round of magnetic separation. Set aside for **5 minutes** and repeat Step 7 for a total of 2 x 5-minute separations.
- Top-up to 10 mL with recommended medium and centrifuge the enriched cells at 250 x g for **10 minutes**. Remove supernatant and resuspend cells in 10 mL of recommended medium. Repeat centrifugation.
- Remove the supernatant and resuspend in 10 mL of recommended medium. Incubate at room temperature (15 - 22°C) for **20 minutes**. *Note: This 20-minute incubation is optional but may improve cell purities and/or recoveries.*
- Centrifuge at 250 x g for **10 minutes**. Resuspend the cells at a concentration of 5×10^7 cells/mL in recommended medium and proceed to CD25⁺ cell selection using the EasySep® Mouse CD25 Positive Selection Kit (Catalog #18761).
Note: If sample contains fewer than 2.5×10^7 cells, resuspend in 500 µL.

Components:

• EasySep [®] Mouse CD4 ⁺ T Cell Pre-Enrichment Cocktail	0.5 mL
• EasySep [®] Biotin Selection Cocktail	2 x 1.0 mL
• EasySep [®] D Magnetic Particles	2 x 1.0 mL
• EasySep [®] Blocking Solution	6 x 1.0 mL
• Normal Rat Serum	2.0 mL



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[®] Mouse CD4⁺ T Cell Pre-Enrichment Cocktail, EasySep[®] Biotin Selection Cocktail and EasySep[®] D Magnetic Particles label non-CD4⁺ T cells for magnetic separation. These reagents are designed to pre-enrich CD4⁺ T cells from mouse spleen cell suspensions by depletion of non-CD4⁺ cells, prior to CD25⁺ cell selection using the EasySep[®] Mouse CD25 Positive Selection Kit (Catalog #18761). For enrichment of all CD4⁺ T cells when not proceeding to CD25⁺ cell selection, we recommend the EasySep[®] Mouse CD4⁺ T Cell Enrichment Kit (Catalog #19752).

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 (reverse side).

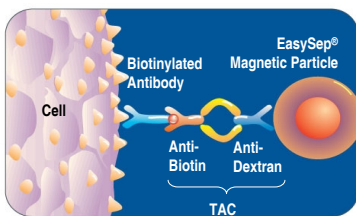


Figure 1.
Schematic Drawing of
EasySep[®] TAC Magnetic
Labeling of Mouse Cells.

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 - 1.5 x 10⁶ nucleated cells/mL in recommended medium. Ammonium chloride treatment is not recommended when preparing the cells for separation.

OPTIMAL CELL NUMBER. The use of fewer than 5 x 10⁷ cells per separation may result in sub-optimal performance.

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

ASSESSING PURITY. Please see the Product Information Sheet for the EasySep[®] Mouse CD25 Selection Kit (Catalog #18761) for details of CD4⁺CD25⁺ cell purity assessment.

COMPONENT DESCRIPTIONS:**EASYSEP[®] MOUSE CD4⁺ T CELL PRE-ENRICHMENT COCKTAIL****CODE #19772C**

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 (CD8, CD11b, CD19, CD24, CD45R, CD49b). 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 IgG₁ 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 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[®] D MAGNETIC PARTICLES**CODE #19250**

A suspension of magnetic dextran iron particles in TRIS buffer.

EASYSEP[®] BLOCKING SOLUTION**CODE #19710**

A blocking solution required for subsequent CD25⁺ cell selection from CD4⁺ T cell pre-enriched cell populations.

NORMAL RAT SERUM**CODE #13551**

This normal rat serum is used to prevent non-specific binding of rat antibodies to mouse cells. Serum has been certified by the manufacturer to be mycoplasma-free.

STABILITY AND STORAGE:**EASYSEP[®] MOUSE CD4⁺ T CELL PRE-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 - 22°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 - 22°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 - 22°C), and should be refrigerated upon receipt.

NORMAL RAT SERUM

Product stable at 2 - 8°C until expiry date as indicated on label. Stable for at least 2 years if stored at -20°C. Contents have been sterility tested.

EASYSEP[®] BLOCKING SOLUTION

Product stable at 2 - 8°C or room temperature (15 - 22°C) until expiry date as indicated on label. Contents have been sterility tested. Please note that repeated exposure to air may cause some crystallization to occur around the edge of the tube. This crystallization does not affect the performance of the blocking solution.