PROCEDURE

EasySep® POSITIVE SELECTION

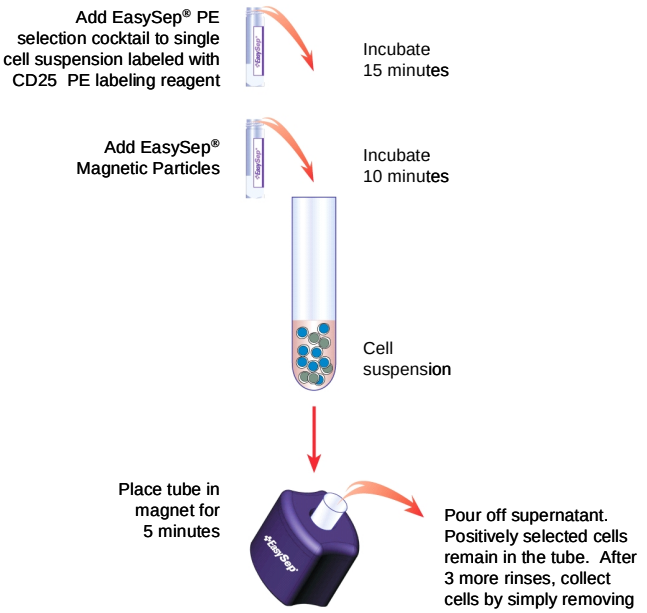
# MOUSE CD25 POSITIVE SELECTION KIT

CATALOG #18721 (18761)

V 1.1.0

THIS PRODUCT INFORMATION SHEET IS PROVIDED FOR USE WITH THE PURPLE EASYSEP® MAGNET. IF YOU WOULD LIKE TO USE THIS PRODUCT WITH "THE BIG EASY" SILVER EASYSEP® MAGNET OR WITH ROBOSEP® - THE FULLY AUTOMATED CELL SEPARATOR, PLEASE CONTACT STEMCELL TECHNOLOGIES FOR SUGGESTIONS.

## MANUAL EASYSEP® PROTOCOL DIAGRAM




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

This procedure is for processing **500 µL – 2 mL** of sample (up to 1 x $10^8$ cells).

1. Prepare a single suspension of $CD4^+$ T cells using the EasySep® Mouse $CD4^+$ T Cell Pre-Enrichment Kit (Catalog #19772). Resuspend enriched cells at a concentration of 5 x $10^7$ cells/mL in recommended medium (see Notes and Tips, reverse side). For samples containing 2.5 x107 cells or fewer, resuspend in 500 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, Catalog #352058) are recommended.*

2. Add CD25 PE Labeling Reagent at **50 µL/mL** of cells (e.g. for 2 mL of cells, add 100 µL of labeling reagent). Mix well and incubate at room temperature for **15** minutes.

3. Add EasySep® PE Selection Cocktail at **33 µL/mL** cells (e.g. for 2 mL of cells, add 66 µL of cocktail). Mix well and incubate at room temperature for **15** minutes.

4. Mix EasySep® Special Application (SA) Magnetic Nanoparticles to ensure that they are in a uniform suspension by pipetting vigorously up and down more than 5 times. Vortexing is not recommended. Add the nanoparticles at **50 µL/mL** of cells (e.g. for 2 mL of cells, add 100 µL of nanoparticles). Mix well and incubate at room temperature for **10** minutes.

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 magnet, and in one continuous motion invert the magnet and tube, pouring off the supernatant fraction containing the $CD4^+CD25^-$ cells into a new 5 mL polystyrene tube (see note below). The magnetically labeled $CD4^+CD25^+$ cells will remain inside the tube, held by the magnetic field of the EasySep® Magnet. Hold 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.

Note: the $CD4^+CD25^{neg}$ cells in the supernatant can be further purified. Place the new tube containing the supernatant from the first separation into the EasySep® magnet, and incubate for 10 minutes. Pour off the supernatant fraction into a new 5 mL tube; these $CD4^+CD25^{neg}$ cells are now ready for use.

7. Remove the tube containing the selected $CD4^+CD25^+$ cells from the magnet and add 2.5 mL of recommended medium. Mix the cell suspension by gently pipetting up and down 2 - 3 times. Place the tube back in the magnet and set aside for **5** minutes.

8. Repeat Steps 6 and 7 twice, and then Step 6 once more, for a total of 4 x 5-minute separations in the magnet. Remove tube from magnet and resuspend cells in an appropriate amount of desired medium. The positively selected $CD4^+CD25^+$ cells are now ready for use.

CATALOG #18721 (18761) For labeling 3 x $10^8$ ($10^9$) total cells

Mouse CD25 Positive Selection Kit Components:

| | |
|---|---|
| • EasySep® PE Selection Cocktail | 1.0 mL |
| • EasySep® Special Application (SA) Magnetic Nanoparticles | 1.0 mL |
| • EasySep® Mouse CD25PE Labeling Reagent | 0.3 mL (1.0 mL) |

EasySep® POSITIVE SELECTION

## REQUIRED EQUIPMENT:

EasySep® Magnet (Catalog #18000), or "The Big Easy" EasySep® Magnet (Catalog #18001), or RoboSep® (Catalog #20000).

## PRODUCT DESCRIPTION AND APPLICATIONS:

EasySep® PE Selection Cocktail and EasySep® Magnetic Nanoparticles are designed to positively select $CD4^+CD25^+$ cells labeled with EasySep® CD25PE labeling reagent from a population of pre-enriched $CD4^+$ T cells. The mouse FcR blocker (anti-CD16/32) included with the PE-labeling reagent prevents nonspecific selection of unwanted cells.

## EASYSEP® LABELING OF HUMAN CELLS:

Cells specifically targeted with PE-labeling reagent are then labeled with EasySep® dextran-coated magnetic nanoparticles using bispecific Tetrameric Antibody Complexes (TAC). These complexes recognize both dextran and the PE molecule on the PE-labeling reagent (Figure 1). Magnetically labeled cells are then separated from unlabeled cells using the EasySep® procedure (reverse side).

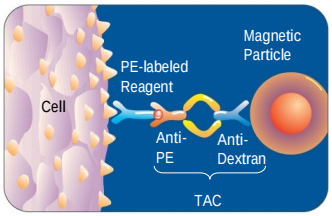



Figure 1.
Schematic Drawing of EasySep® TAC Magnetic Labeling of Mouse Cells.

## NOTES AND TIPS:

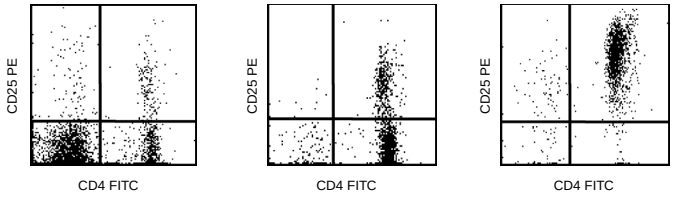
**PREPARING A SINGLE CELL SUSPENSION.** This product is designed to positively select $CD4^+CD25^+$ cells from a population of pre-enriched $CD4^+$ T cells. Prepare cells using the EasySep® Mouse $CD4^+$ T Cell Pre-Enrichment Kit (Catalog #19772) according to the instructions on the product information sheet.

**RECOMMENDED MEDIUM.** Phosphate Buffered Saline (PBS) + 2% Fetal Bovine Serum (FBS) (Catalog #07905) with 1 mM EDTA. Medium should be $Ca^{++}$ and $Mg^{++}$ free.

**ASSESSING PURITY.** Purity of $CD4^+CD25^+$ T cells can be measured by flow cytometry after staining with a fluorochrome-conjugated anti-CD4 antibody (e.g. FITC anti-CD4, Catalog #10701); the positively selected $CD25^+$ cells have already been PE-labeled.

## TYPICAL EASYSEP® $CD4^+CD25^+$ PE SELECTION PROFILE

| **Start:** | **$CD4^+$ T Cell Pre-Enriched:** | **$CD25^+$ Selected:** |
|---|---|---|
| 22.3% $CD4^+$ cells | 93.5% $CD4^+$ cells | |
| 3.6% $CD4^+CD25^+$ cells | 16.7% $CD4^+CD25^+$ cells | 89.6% $CD4^+CD25^+$ cells |




The $CD4^+CD25^+$ cell content of the selected cells typically ranges from 85 - 97%.

## COMPONENT DESCRIPTIONS:

**EASYSEP® PE SELECTION COCKTAIL** **CODE #18151**

This cocktail contains a combination of monoclonal antibodies purified from hybridoma culture supernatant by affinity chromatography using Protein A or Protein G Sepharose. These antibodies are bound in bispecific Tetrameric Antibody Complexes (TAC) which are directed against PE (Phycoerythrin) and dextran. The mouse monoclonal antibody subclass is $IgG_1$. This cocktail is supplied in PBS with 0.1% Bovine Serum Albumin (BSA). 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® SA MAGNETIC NANOPARTICLES** **CODE #18250**

A suspension of magnetic dextran iron particles in water.

**EASYSEP® MOUSE CD25PE LABELING REAGENT** **CODE #18721C (18761C)**

Supplied in PBS with 0.1% BSA and 0.1% sodium azide. Contains an antibody directed against mouse CD16/32 ($Fc\gamma$ III/II receptor).

## STABILITY AND STORAGE:

**EASYSEP® PE 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® SA 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.

**EASYSEP® MOUSE CD25 PE LABELING REAGENT**

Product stable at 2 - 8°C until expiry date as indicated on label. Protect from light. 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.

***Hazardous Ingredient: Sodium Azide.*** *Avoid exposure to skin and eyes, ingestion and contact with heat, acids and metals. Wash exposed skin with soap and water. Flush eyes with water. Dilute with running water before discharging into plumbing.*

See Material Safety Data Sheet for more information.



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