

**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™.**

If using other EasySep™ Magnets, please visit [www.stemcell.com](http://www.stemcell.com) to download the magnet-specific Product Information Sheet or contact STEMCELL Technologies' Technical Support at [techsupport@stemcell.com](mailto:techsupport@stemcell.com).

#### A) MANUAL 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).

1. Prepare cell suspension at a concentration of  $1 - 1.5 \times 10^8$  cells/mL in recommended medium (see Notes and Tips, reverse side). Cells must be placed in a 5 mL (12 x 75 mm) polystyrene tube to properly fit into the Purple EasySep™ Magnet. Add the Normal Rat Serum (provided) at **50 µL/mL** of cells (e.g. for 1 mL of cell suspension, add 50 µL of rat serum).  
*Falcon® 5 mL Polystyrene Round-Bottom Tubes (Corning® Catalog #352058) are recommended.*
2. Add the EasySep™ Mouse CD4+ T Cell Pre-Enrichment 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 the EasySep™ Biotin Selection Cocktail at **150 µL/mL of cells** (e.g. for 1 mL of cells, add 150 µL of 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 a uniform suspension with no visible aggregates.
5. Add the EasySep™ D Magnetic Particles at **150 µL/mL of cells** (e.g. for 1 mL of cells, add 150 µL of magnetic particles). Mix well and incubate at room temperature (15 - 25°C) for **10 minutes**.
6. 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 at room temperature (15 - 25°C) 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 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.
8. Add **200 µL** of EasySep™ Blocking Solution (provided) to the cell suspension in the new tube. Cap tube and vortex for 3 seconds.
9. 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 at room temperature (15 - 25°C) for **5 minutes** and repeat Step 7 for a total of 2 x 5-minute separations.
10. Top-up the new tube to 4 mL with recommended medium and centrifuge the enriched cells at  $250 \times g$  for **10 minutes**. Remove supernatant and resuspend cells in 4 mL of recommended medium. Repeat centrifugation.
11. Remove the supernatant and resuspend in 4 mL of recommended medium. Incubate at room temperature (15 - 25°C) for **20 minutes**. *Note: This 20-minute incubation is optional but may improve cell purities and/or recoveries.*
12. Centrifuge at  $250 \times g$  for **10 minutes**. Resuspend the cells at a concentration of  $5 \times 10^7$  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  $2.5 \times 10^7$  cells, resuspend in 500 µL of medium.*

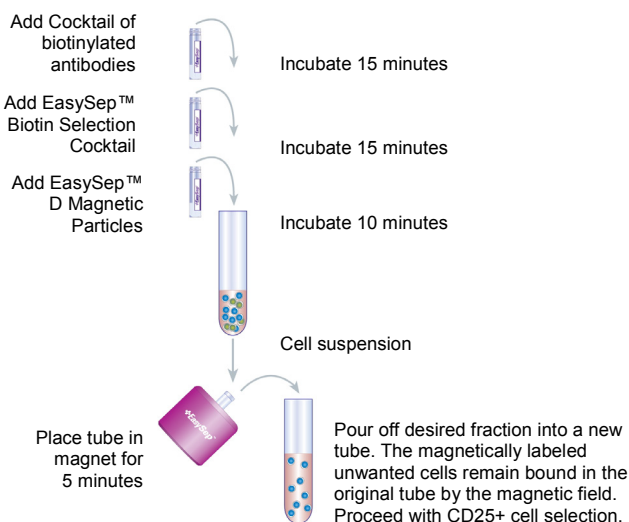
#### B) MANUAL EASYSEP™ PROTOCOL USING “THE BIG EASY” SILVER EASYSEP™ MAGNET (CATALOG #18001)

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

1. Prepare cell suspension at a concentration of  $1 - 1.5 \times 10^8$  cells/mL in recommended medium (see Notes and Tips, reverse side). Cells must be placed in a 14 mL (17 x 100 mm) polystyrene tube to properly fit into the Silver EasySep™ Magnet. Add the Normal Rat Serum (provided) at **50 µL/mL** of cells (e.g. for 2 mL of cell suspension, add 100 µL of rat serum).  
*Falcon® 14 mL Polystyrene Round-Bottom Tubes (Corning® Catalog #352057) are recommended.*
2. Add the EasySep™ Mouse CD4+ T Cell Pre-Enrichment 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 the EasySep™ Biotin Selection Cocktail at **150 µL/mL of cells** (e.g. for 2 mL of cells, add 300 µL of 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 a uniform suspension with no visible aggregates.
5. Add the EasySep™ D Magnetic Particles at **150 µL/mL of cells** (e.g. for 2 mL of cells, add 300 µL of magnetic particles). Mix well and incubate at room temperature (15 - 25°C) for **10 minutes**.
6. Bring the cell suspension up 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 at room temperature (15 - 25°C) 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 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.
8. 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.
9. 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 at room temperature (15 - 25°C) for **5 minutes** and repeat Step 7 for a total of 2 x 5-minute separations.
10. Top-up to 10 mL with recommended medium and centrifuge the enriched cells at  $250 \times g$  for **10 minutes**. Remove supernatant and resuspend cells in 10 mL of recommended medium. Repeat centrifugation.
11. Remove the supernatant and resuspend in 10 mL of recommended medium. Incubate at room temperature (15 - 25°C) for **20 minutes**. *Note: This 20-minute incubation is optional but may improve cell purities and/or recoveries.*
12. Centrifuge at  $250 \times g$  for **10 minutes**. Resuspend the cells at a concentration of  $5 \times 10^7$  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  $2.5 \times 10^7$  cells, resuspend in 500 µL.*

#### MANUAL EASYSEP™ PROTOCOL DIAGRAM



STEMCELL TECHNOLOGIES INC.'S QUALITY MANAGEMENT SYSTEM IS CERTIFIED TO ISO 13485 MEDICAL DEVICE STANDARDS.  
FOR RESEARCH USE ONLY. NOT INTENDED FOR HUMAN OR ANIMAL DIAGNOSTIC OR THERAPEUTIC USES.



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VERSION 2.1.0  
DOCUMENT #29124

**Components:**

• EasySep™ Mouse CD4+ T Cell Pre-Enrichment Cocktail	0.5 mL
• EasySep™ Biotin Selection Cocktail	2 x 1 mL
• EasySep™ D Magnetic Particles	2 x 1 mL
• Normal Rat Serum	2 mL
• EasySep™ Blocking Solution	6 x 1 mL



NEGATIVE SELECTION

**REQUIRED EQUIPMENT:**

EasySep™ Magnet (Catalog #18000), or “The Big Easy” EasySep™ Magnet (Catalog #18001).

**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+ T 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 Isolation Kit (Catalog #19852).

**NOTES AND TIPS:**

**PREPARING A SINGLE CELL SUSPENSION** Disrupt spleen in phosphate-buffered saline (PBS) or Hanks' balanced salt solution (HBSS) plus 2% fetal bovine serum (FBS). Remove clumps and debris by passing cell suspension through a 70 µm mesh nylon strainer. Centrifuge at 300 x g for 10 minutes and resuspend at 1 x 10<sup>8</sup> 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 10<sup>7</sup> cells per separation may result in sub-optimal performance.

**RECOMMENDED MEDIUM** The recommended medium is EasySep™ Buffer (Catalog #20144), or PBS + 2% FBS with 1 mM EDTA. HBSS can be used in place of PBS. Medium should be Ca<sup>2+</sup> and Mg<sup>2+</sup>.

**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 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 IgG<sub>1</sub> monoclonal antibodies bound in bispecific Tetrameric Antibody Complexes by rat monoclonal antibodies against mouse IgG. 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.

**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.

**EASYSEP™ BLOCKING SOLUTION CODE #19710**

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

**STABILITY AND STORAGE:****EASYSEP™ MOUSE CD4+ T CELL PRE-ENRICHMENT COCKTAIL****EASYSEP™ BIOTIN SELECTION COCKTAIL****EASYSEP™ D MAGNETIC PARTICLES**

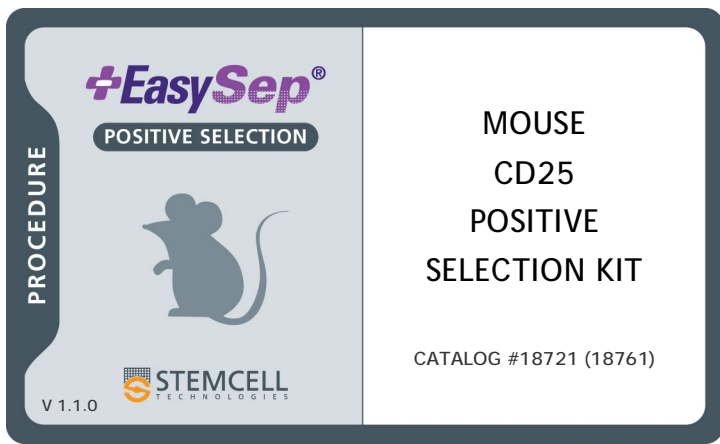
Product stable at 2 - 8°C until expiry date as indicated on label. Contents have been sterility-tested. Do not freeze this product. This product may be shipped at room temperature (15 - 25°C), and should be refrigerated upon receipt.

**NORMAL RAT SERUM**

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

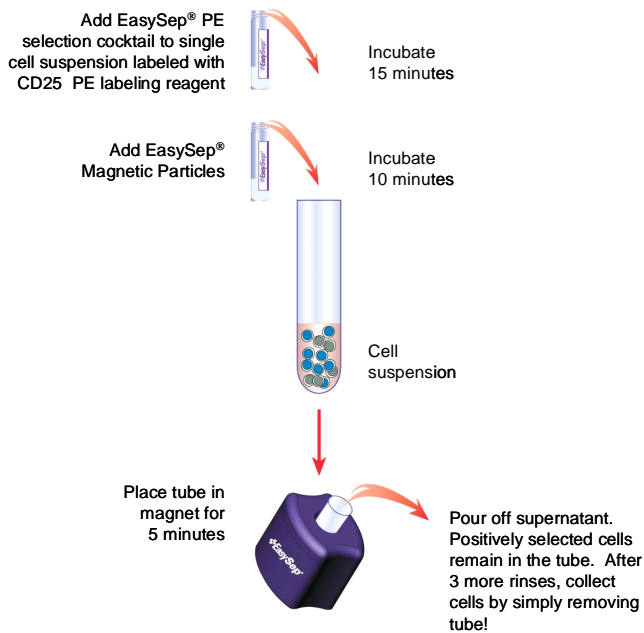
**EASYSEP™ BLOCKING SOLUTION**

Product stable at 2 - 8°C or room temperature (15 - 22°C) until expiry date as indicated on label. 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.



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  $1 \times 10^8$  cells).

1. Prepare a single suspension of CD4<sup>+</sup> T cells using the EasySep® Mouse CD4<sup>+</sup> T Cell Pre-Enrichment Kit (Catalog #19772). Resuspend enriched cells at a concentration of  $5 \times 10^7$  cells/mL in recommended medium (see Notes and Tips, reverse side). For samples containing  $2.5 \times 10^7$  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<sup>+</sup>CD25<sup>-</sup> cells into a new 5 mL polystyrene tube (see note below). The magnetically labeled CD4<sup>+</sup>CD25<sup>+</sup> 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<sup>+</sup>CD25<sup>neg</sup> 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<sup>+</sup>CD25<sup>neg</sup> cells are now ready for use.

7. Remove the tube containing the selected CD4<sup>+</sup>CD25<sup>+</sup> 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<sup>+</sup>CD25<sup>+</sup> cells are now ready for use.

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)



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<sup>+</sup>CD25<sup>+</sup> cells labeled with EasySep® CD25PE labeling reagent from a population of pre-enriched CD4<sup>+</sup> 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).

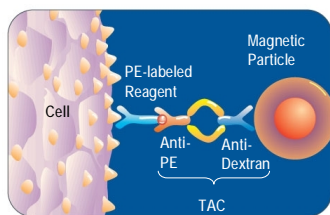


Figure 1. Schematic Drawing of EasySep® TAC Magnetic Labeling of Mouse Cells.

NOTES AND TIPS:

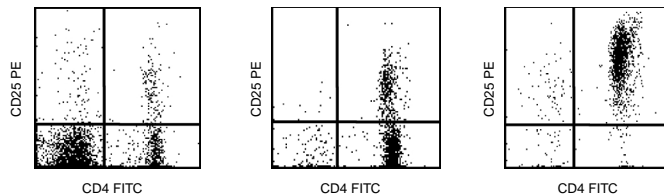
**PREPARING A SINGLE CELL SUSPENSION.** This product is designed to positively select CD4<sup>+</sup>CD25<sup>+</sup> cells from a population of pre-enriched CD4<sup>+</sup> T cells. Prepare cells using the EasySep® Mouse CD4<sup>+</sup> T Cell Pre-Enrichment Kit (Catalog #19772) according to the instructions 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<sup>2+</sup> and Mg<sup>2+</sup> free.

**ASSESSING PURITY.** Purity of CD4<sup>+</sup>CD25<sup>+</sup> 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<sup>+</sup> cells have already been PE-labeled.

TYPICAL EASYSEP® CD4<sup>+</sup>CD25<sup>+</sup> PE SELECTION PROFILE

Start:	CD4 <sup>+</sup> T Cell Pre-Enriched:	CD25 <sup>+</sup> Selected:
22.3% CD4 <sup>+</sup> cells 3.6% CD4 <sup>+</sup> CD25 <sup>+</sup> cells	93.5% CD4 <sup>+</sup> cells 16.7% CD4 <sup>+</sup> CD25 <sup>+</sup> cells	89.6% CD4 <sup>+</sup> CD25 <sup>+</sup> cells



The CD4<sup>+</sup>CD25<sup>+</sup> 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<sub>1</sub>. 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 (Fcγ 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.