PROCEDURE

EasySep™

POSITIVE SELECTION

HUMAN TH1 CELL ISOLATION KIT

CATALOG #18161

PRODUCT DESCRIPTION

The EasySep™ Human Th1 Cell Isolation Kit is a two-step method designed to enrich human T helper type 1 (Th1) cells. Th1 (CD4+CXCR3+) cells can be isolated from fresh peripheral blood mononuclear cells (PBMCs) and leukapheresis samples. First, non-CD4+ T cells are depleted using the EasySep™ CD4+CXCR3+ T Cell Pre-Enrichment Cocktail (Catalog #19161C). Next, the CD4+CXCR3+ T cells are isolated using the EasySep™ Human CXCR3 Positive Selection Cocktail (Catalog #18361C).

This kit is compatible for use with RoboSep™ **(Section A, page 1)**, the Purple EasySep™ Magnet **(Section B, page 2)**, and "The Big Easy" Silver EasySep™ Magnet **(Section C, page 2)**.

PLEASE NOTE: Two different magnetic particles are provided in the kit – EasySep™ D2 Magnetic Particles (Catalog #19650) and EasySep™ Magnetic Nanoparticles (Catalog #18150H) **(brown ●).** It is important to follow the procedures outlined in this Product Information Sheet carefully and use the EasySep™ D2 Magnetic Particles only for the EasySep™ Human CD4+CXCR3+ T Cell Pre-Enrichment protocol and the EasySep™ Magnetic Nanoparticles **(brown ●)** only for the EasySep™ Human CXCR3 Positive Selection protocol. Please contact STEMCELL Technologies' Technical Support at techsupport@stemcell.com for more information.

SECTION A:

FULLY AUTOMATED PROTOCOL USING ROBOSEP™ (CATALOG #20000).

This procedure is used for processing **0.5 mL - 8 mL** of sample (up to 4 x 10^8 cells).

I. RoboSep™ Human CD4+CXCR3+ T Cell Pre-Enrichment

1. Prepare a cell suspension at a concentration of 5 x 10^7 cells/mL in RoboSep™ Buffer (Catalog #20104). Cells must be placed in a 14 mL (17 x 100 mm) polystyrene tube to properly fit into the RoboSep™ carousel.
   Falcon™ 14 mL Polystyrene Round-Bottom Tubes (BD Biosciences, Catalog #352057) are recommended.
2. Select the appropriate RoboSep™ protocol:
   - Human Th1 Pre-Enrichment 18161 (19161C)

   If a modified RoboSep™ protocol is required, please contact STEMCELL Technologies' Technical Support at techsupport@stemcell.com.
3. Vortex the EasySep™ D2 Magnetic Particles for 30 seconds before loading. Ensure that the particles are in a uniform suspension with no visible aggregates.
4. Load the RoboSep™ carousel as directed by the on-screen prompts. When all desired quadrants are loaded, press the green "Run" button. All cell labeling and separation steps will be performed by RoboSep™.
5. When cell separation is complete, remove the tube containing the pre-enriched cells from the RoboSep™ carousel. The pre-enriched cells are located in the 14 mL tube in the Q2 quadrant.
6. Centrifuge the pre-enriched cells at 300 x *g* for **10 minutes** at room temperature (15 - 25°C). Carefully remove the supernatant and resuspend the cell pellet in RoboSep™ Buffer according to **Table 1** (page 3). The sample is now ready for the RoboSep™ Human CXCR3 Positive Selection protocol (**Section A part II**).

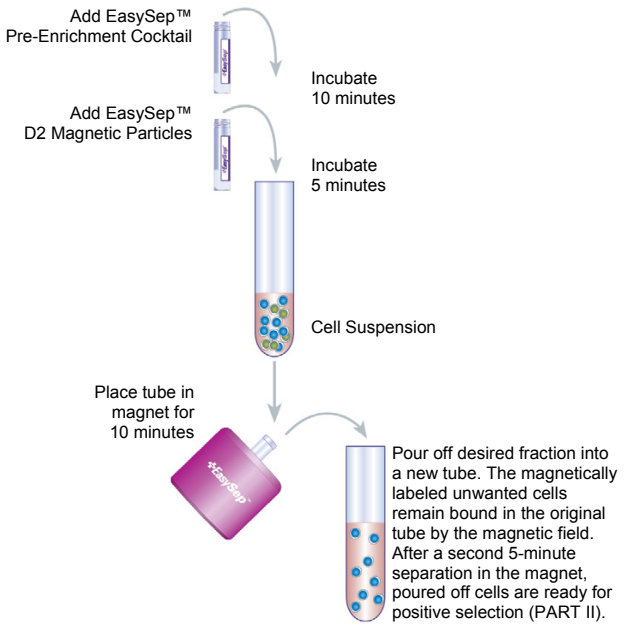
II. RoboSep™ Human CXCR3 Positive Selection

1. Select the appropriate RoboSep™ protocol:
   - Human CXCR3 Positive Selection 18161 (18361C)

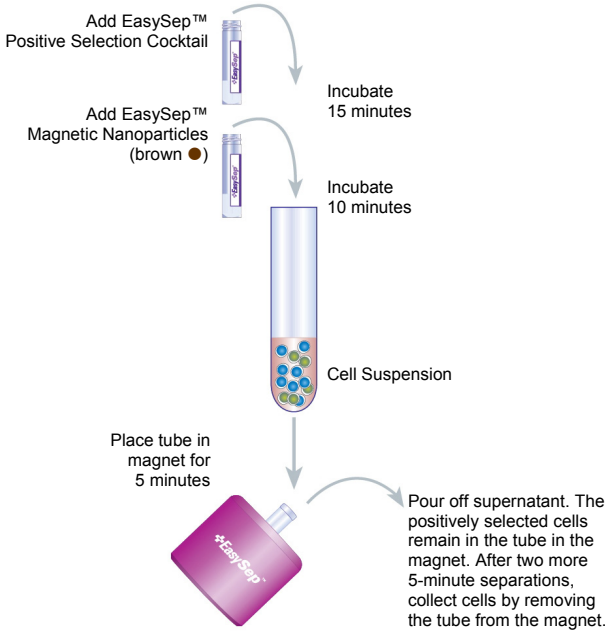
   If a modified RoboSep™ protocol is required, please contact STEMCELL Technologies' Technical Support at techsupport@stemcell.com.
2. Mix the EasySep™ Magnetic Nanoparticles **(brown ●)** by vigorously pipetting up and down more than 5 times before loading. Ensure that the particles are in a uniform suspension. Vortexing is not recommended.
3. Load the RoboSep™ carousel as directed by the on-screen prompts. When all desired quadrants are loaded, press the green "Run" button. All cell labeling and separation steps will be performed by RoboSep™.
4. When cell separation is complete, remove the tube containing the isolated cells from the magnet. Resuspend in desired medium. The positively selected cells are now ready for use.

MANUAL EASYSEP™ PROTOCOL DIAGRAM

PART I: CD4+CXCR3+ T CELL PRE-ENRICHMENT



PART II: CXCR3 POSITIVE SELECTION



SECTION B:

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

I. EasySep™ Human CD4+CXCR3+ T Cell Pre-Enrichment

This procedure is used for processing **0.25 mL - 2 mL** of sample (up to 1 x 10^8 cells).

1. Prepare a cell suspension at a concentration of 5 x 10^7 cells/mL in recommended medium (see Notes and Tips, page 3). 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 Biosciences, Catalog #352058) are recommended.
2. Add the EasySep™ Human CD4+CXCR3+ T Cell Pre-Enrichment Cocktail at **50 µL/mL cells** (e.g. for 2 mL of cells, add 100 µL of cocktail). Mix well and incubate at room temperature (15 - 25°C) for **10 minutes**.
3. Vortex the EasySep™ D2 Magnetic Particles for 30 seconds. Ensure that the particles are in a uniform suspension with no visible aggregates.
4. Add the D2 Magnetic Particles at **75 µL/mL cells** (e.g. for 2 mL of cells, add 150 µL of particles). Mix well and incubate at room temperature (15 - 25°C) for **5 minutes**.
5. 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 and incubate at room temperature (15 - 25°C) for **10 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 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.*
7. Remove the original tube from the EasySep™ Magnet. Place the new tube containing the poured-off cells into the magnet and set aside for **5 minutes**.
8. Repeat Step 6 for a total of two separations in the magnet (**1 x 10 minutes, 1 x 5 minutes**).
9. Centrifuge pre-enriched cells in the new tube at 300 x *g* for **10 minutes** at room temperature (15 - 25°C). Carefully remove supernatant and resuspend cell pellet in **0.25 mL** of the recommended medium **Table 1** (page 3) and continue with the EasySep™ Human CXCR3 Positive Selection protocol **(Section B part II, below)**.

II. EasySep™ Human CXCR3 Positive Selection

This procedure is used for processing **0.25 - 1 mL** of pre-enriched cells prepared with EasySep™ Human CD4+CXCR3+ T Cell Pre-Enrichment protocol **(Section B part I, above).**

1. Add the EasySep™ Human CXCR3 Positive Selection Cocktail at **50 µL/mL of cells**. Mix well and incubate at room temperature (15 - 25°C) for **15 minutes**.
2. Mix the EasySep™ Magnetic Nanoparticles **(brown ●)** by vigorously pipetting up and down more than 5 times. Ensure that the particles are in a uniform suspension. *Vortexing is not recommended.*
3. Add the nanoparticles at **100 µL/mL of cells**. Mix well and incubate at room temperature (15 - 25°C) for **10 minutes**.
4. 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**.
5. Pick up the EasySep™ Magnet, and in one continuous motion invert the magnet and tube, pouring off the supernatant fraction into a new 5 mL polystyrene tube. The magnetically labeled cells will remain inside the 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.*
6. Remove the tube 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**.
7. Repeat Steps 5 and 6, then Step 5 once more, for a total of **3 x 5-minute** separations in the magnet. Remove the tube from the magnet and resuspend cells in desired medium. The positively selected cells are now ready for use.

SECTION C:

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

I. EasySep™ Human CD4+CXCR3+ T Cell Pre-Enrichment

This procedure is used for processing **0.5 mL - 8 mL** of sample (up to 4 x 10^8 cells).

1. Prepare a cell suspension at a concentration of 5 x 10^7 cells/mL in recommended medium (see Notes and Tips, page 3). Cells must be placed in a 14 mL (12 x 75 mm) polystyrene tube to properly fit into the Silver EasySep™ Magnet.
   Falcon™ 14 mL Polystyrene Round-Bottom Tubes (BD Biosciences, Catalog #352057) are recommended.
2. Add the EasySep™ Human CD4+CXCR3+ 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 **10 minutes**.
3. Vortex the EasySep™ D2 Magnetic Particles for 30 seconds. Ensure that the particles are in a uniform suspension with no visible aggregates.
4. Add the D2 Magnetic Particles at **75 µL/mL cells** (e.g. for 2 mL of cells, add 150 µL of particles). Mix well and incubate at room temperature (15 - 25°C) for **5 minutes**.
5. Bring the cell suspension up to a total volume of **5 mL** (for ≤2 x 10^8 cells) or to **10 mL** (for >2.0 - 4.0 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 and incubate at room temperature (15 - 25°C) for **10 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 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.*
7. Remove the original tube from the EasySep™ Magnet. Place the new tube containing the poured-off cells into the magnet and set aside for **5 minutes**.
8. Repeat Step 6 for a total of two separations in the magnet (**1 x 10 minutes, 1 x 5 minutes**).
9. Centrifuge pre-enriched cells in the new tube at 300 x *g* for **10 minutes** at room temperature (15 - 25°C). Carefully remove the supernatant and resuspend the cell pellet in the recommended medium according to **Table 1** (page 3) and continue with the EasySep™ Human CXCR3 Positive Selection protocol **(Section C part II, below)**.

II. EasySep™ Human CXCR3 Positive Selection

This procedure is used for processing **0.25 - 2 mL** of pre-enriched cells prepared with EasySep™ Human CD4+CXCR3+ T Cell Pre-Enrichment Cocktail protocol **(Section C part I, above)**

1. Add the EasySep™ Human CXCR3 Positive Selection Cocktail at **50 µL/mL of cells**. Mix well and incubate at room temperature (15 - 25°C) for **15 minutes**.
2. Mix the EasySep™ Magnetic Nanoparticles **(brown ●)** by vigorously pipetting up and down more than 5 times. Ensure that the particles are in a uniform suspension. *Vortexing is not recommended.*
3. Add the nanoparticles at **100 µL/mL of cells**. Mix well and incubate at room temperature (15 - 25°C) for **10 minutes**.
4. 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**.
5. Pick up the EasySep™ Magnet, and in one continuous motion invert the magnet and tube, pouring off the supernatant fraction into a new 14 mL polystyrene tube. The magnetically labeled cells will remain inside the 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.*
6. Remove the tube 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**.
7. Repeat Steps 5 and 6, then Step 5 once more, for a total of **3 x 5-minute** separations in the magnet. Remove the tube from the magnet and resuspend cells in desired medium. The positively selected cells are now ready for use.

REQUIRED EQUIPMENT:

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

EASYSEP™ LABELING OF HUMAN CELLS:

Target cells are specifically labeled with dextran-coated magnetic particles using bispecific Tetrameric Antibody Complexes (TACs). These complexes recognize both dextran and the target cell surface antigen (Figure 1). Magnetically labeled cells are then separated from unlabeled cells using the EasySep™ procedure.

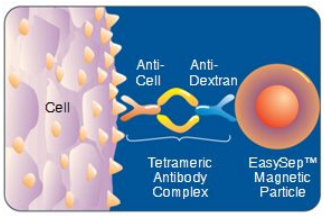


Figure 1.
Schematic Drawing of EasySep™ TAC Magnetic Labeling of Human Cells.

NOTES AND TIPS:

PREPARING THE CELL SUSPENSION

From whole peripheral blood

Prepare a mononuclear cell suspension from fresh whole peripheral blood by density gradient centrifugation.

From peripheral blood apheresis (leukopak)

If working with large volumes (>150 mL), concentrate Leukopak cells first by centrifuging at 500 x *g* for 10 minutes. Remove the supernatant and resuspend the cells in 1/10th of the original Leukopak volume with recommended medium (e.g. for 300 mL of cells, resuspend in 30 mL of recommended medium). For small volumes (150 mL or less), add the Ammonium Chloride Solution (Catalog #07800/07850) directly to the cell suspension.

1. Add an equal volume of Ammonium Chloride Solution to the Leukopak suspension (e.g. for 5 mL of Leukopak suspension, add 5 mL Ammonium Chloride Solution).
2. Incubate 15 minutes on ice.
3. Centrifuge at 300 x *g* for 10 minutes at room temperature (15 - 25°C). Remove the supernatant.
4. Wash the cells by topping up the tube with recommended medium. Centrifuge the cells at 150 x *g* for 10 minutes at room temperature (15 - 25°C) with the brake off. Carefully remove the supernatant.
5. Repeat the wash step one or more times until most of the platelets have been removed (indicated by a clear supernatant).
6. Resuspend cells at recommended cell concentration, in the recommended medium.

From Frozen peripheral blood apheresis (leukopak)

Newly thawed cells show a reduction in CXCR3 expression. A decrease in the production of IFN-γ is also seen in isolated CD4+CXCR3+ T cells after stimulation with PMA-Ionomycin. Culturing thawed cells overnight prior to isolation may be required to obtain optimal results. Please contact STEMCELL Technologies' Technical Support at techsupport@stemcell.com if you wish to isolate CD4+CXCR3+ T cells from previously frozen PBMCs.

RECOMMENDED MEDIUM The recommended medium is RoboSep™ Buffer (Catalog #20104), or phosphate-buffered saline (PBS) + 2% fetal bovine serum (FBS) (Catalog #07905) with 1 mM EDTA. Medium should be Ca++ and Mg++ free.

ASSESSING PURITY The purity of Th1 (CD4+CXCR3+) cells can be measured by flow cytometry after staining with fluorochrome-conjugated anti-CD4, anti-CD45RA and anti-CD183 (CXCR3) antibodies.

The EasySep™ Human CXCR3 Positive Selection cocktail may block some antibody clones used to assess purity by flow cytometry. CD183 (CXCR3) antibody clone: G025H7 is recommended at a concentration of 4.0 µg/mL. Alternatively, positively selected cells can be detected using a fluorescently labeled anti-dextran antibody (e.g. Anti-Dextran Antibody, Clone DX1, FITC; Catalog #60026FI).

In addition, intracellular staining of IFN-γ may be used to assess purity, after stimulation of isolated CD4+CXCR3+ T cells with PMA-Ionomycin.

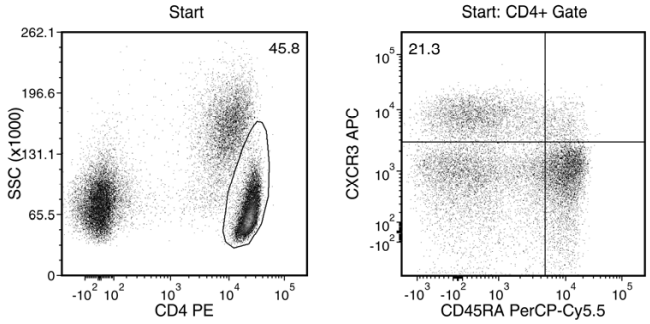
TABLE 1. RECOMMENDED RESUSPENSION VOLUMES FOR EASYSEP™ CXCR3 POSITIVE SELECTION PROTOCOL

MAGNET PLATFORM	CD4+CXCR3+ T CELL PRE-ENRICHMENT		CXCR3+ SELECTION
	START CELL VOLUME	START CELL NUMBER	RESUSPENSION VOLUME
Purple	0.25 - <2.0 mL	0.125 - <1 x 10^8	0.25 mL
Silver	0.5 - < 2.0 mL	0.25 - < 1 x 10^8	0.25 mL
	2 - 4 mL	1 x 10^8 - 2 x 10^8	0.5 mL
	>4 - 8 mL	>2 x 10^8 - 4 x 10^8	1 mL
RoboSep™	0.5 - 4 mL	0.25 X 10^7 - 2 x 10^8	0.5 mL
	>4 - 8 mL	>2 x 10^8 - 4 x 10^8	1 mL

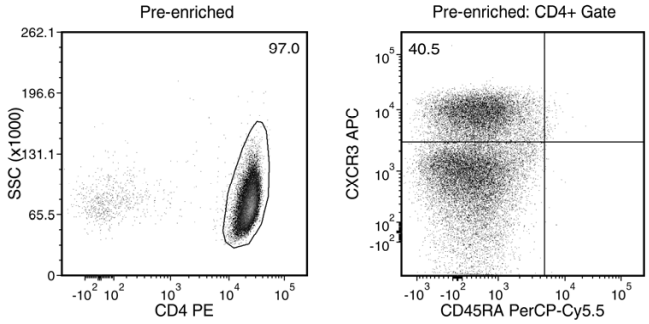
Note: If cells are counted after the CD4+CXCR3+ pre-enrichment protocol, resuspend cells at a concentration of 1 - 5 x 10^7 cells/mL.

TYPICAL EASYSEP™ HUMAN TH1 CELL ISOLATION PROFILE:

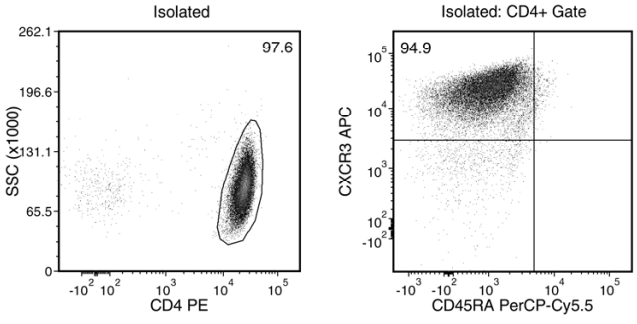
START SAMPLE



EASYSEP™ HUMAN CD4+CXCR3+ T CELL PRE-ENRICHMENT



EASYSEP™ HUMAN CXCR3 POSITIVE SELECTION



Starting with fresh PBMCs, the Th1 (CD4+CXCR3+) cell content of the isolated fraction typically ranges from 85 - 95%. In the example above, the final purities of the start, pre-enriched and isolated fractions are 9.6%, 39.3% and 92.6%, respectively. IFN-γ producing cells in the isolated CD4+CXCR3+ T cell population after stimulation typically ranges from 45 - 80% (data not shown). These values vary amongst donors.

CATALOG #18161

For labeling 10^9 total cells

Components:

• EasySep™ Human CD4+CXCR3+ T Cell Pre-Enrichment Cocktail	1 mL
• EasySep™ D2 Magnetic Particles	2 x 1 mL
• EasySep™ Human CXCR3 Positive Selection Cocktail	0.5 mL
• EasySep™ Magnetic Nanoparticles Positive Selection (brown ●)	1 mL

COMPONENT DESCRIPTIONS:

EASYSEP™ HUMAN CD4+CXCR3+ T CELL PRE-ENRICHMENT COCKTAIL

CODE #19161C

This cocktail contains a combination of monoclonal antibodies bound in bispecific TACs which are directed against cell surface antigens on human blood cells (CD8, CD14, CD16, CD19, CD20, CD36, CD45RA, CD56, CD66b, CD123, TCRγ/δ, glycophorin A) and dextran. The mouse monoclonal antibody subclass is 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™ D2 MAGNETIC PARTICLES

CODE #19650

A suspension of magnetic dextran iron particles in TRIS buffer.

EASYSEP™ HUMAN CXCR3 POSITIVE SELECTION COCKTAIL

CODE #18361C

This cocktail contains a combination of monoclonal antibodies bound in bispecific TACs which are directed against CD183 (CXCR3) and dextran. The mouse monoclonal antibody subclass is IgG_1. This cocktail is supplied in PBS and contains an antibody against human Fc receptor. 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 POSITIVE SELECTION (brown ●)

CODE #18150H

A suspension of magnetic dextran iron nanoparticles in water.

STABILITY AND STORAGE:

EASYSEP™ HUMAN CD4+CXCR3+ T CELL PRE-ENRICHMENT COCKTAIL
EASYSEP™ D2 MAGNETIC PARTICLES
EASYSEP™ HUMAN CXCR3 POSITIVE SELECTION COCKTAIL
EASYSEP™ MAGNETIC NANOPARTICLES POSITIVE SELECTION (brown ●)

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.

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