



## EasySep™ Release Human CD3 Positive Selection Kit

Positive Selection

Catalog #17751

For processing  $1 \times 10^9$  cells



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## Description

Isolate highly purified CD3+ cells from fresh or previously frozen peripheral blood mononuclear cells (PBMCs) or washed leukapheresis samples.

- Highly purified human CD3+ cells isolated in less than 30 minutes
- No-wash removal of EasySep™ Releasable RapidSpheres™

This kit targets CD3+ cells for positive selection with antibody complexes recognizing the CD3 surface marker and EasySep™ Releasable RapidSpheres™. Desired cells are labeled with antibodies and magnetic particles, and separated without columns using an EasySep™ magnet. Unwanted cells are simply poured off, while desired cells remain in the tube. Then, bound magnetic particles are removed from the EasySep™-isolated CD3+ cells, which are immediately available for downstream applications such as flow cytometry, culture, or DNA/RNA extraction. Following cell isolation with this EasySep™ Release kit, antibody complexes remain bound to the cell surface and may interact with Brilliant Violet™ antibody conjugates, polyethylene glycol-modified proteins, or other chemically related ligands.

## Component Descriptions

| COMPONENT NAME                                         | COMPONENT # | QUANTITY | STORAGE                          | SHELF LIFE                               | FORMAT                                                                                                 |
|--------------------------------------------------------|-------------|----------|----------------------------------|------------------------------------------|--------------------------------------------------------------------------------------------------------|
| EasySep™ Release Human CD3 Positive Selection Cocktail | 17751C      | 1 x 1 mL | Store at 2 - 8°C. Do not freeze. | Stable until expiry date (EXP) on label. | A combination of monoclonal antibodies in PBS and 0.1% BSA. Includes an Fc receptor blocking antibody. |
| EasySep™ Releasable RapidSpheres™ 50201                | 50201       | 1 x 1 mL | Store at 2 - 8°C. Do not freeze. | Stable until expiry date (EXP) on label. | A suspension of magnetic particles in water.                                                           |
| EasySep™ Release Buffer                                | 20145       | 1 x 1 mL | Store at 2 - 8°C. Do not freeze. | Stable until expiry date (EXP) on label. | A buffer for release of Releasable RapidSpheres™ from cells following positive selection.              |

BSA - bovine serum albumin; PBS - phosphate-buffered saline

Components may be shipped at room temperature (15 - 25°C) but should be stored as indicated above.

## Sample Preparation

For available fresh and frozen samples, see [www.stemcell.com/primarycells](http://www.stemcell.com/primarycells).

### PERIPHERAL BLOOD

Prepare a PBMC suspension from whole blood by centrifugation over a density gradient medium (e.g. Lymphoprep™, Catalog #07801). For more rapid PBMC preparation, use the SepMate™ RUO (Catalog #86450/86415) or SepMate™ IVD\* (Catalog #85450/85415) cell isolation tube.

If using previously frozen PBMCs, incubate the cells with DNase I Solution (Catalog #07900) at a concentration of 100 µg/mL at room temperature (15 - 25°C) for at least 15 minutes prior to labeling and separation. Filter clumpy suspensions through a 40 µm Cell Strainer (Catalog #27305) for optimal results.

After preparation, resuspend cells at  $1 \times 10^8$  cells/mL in recommended medium.

\* SepMate™ IVD is only available in select regions where it is registered as an In Vitro Diagnostic (IVD) device for the isolation of mononuclear cells (MNCs) from whole blood or bone marrow by density gradient centrifugation. In all other regions SepMate™ is available for research use only (RUO).

### LEUKAPHERESIS (LEUKO PAK)

Wash the peripheral blood leukapheresis sample by adding an equivalent volume of recommended medium or PBS containing 2% fetal bovine serum (FBS). Centrifuge at 500 x g for 10 minutes at room temperature (15 - 25°C). If red blood cell (RBC) lysis is necessary, lyse with Ammonium Chloride Solution (Catalog #07800). If platelet removal is necessary, centrifuge at 120 x g for 10 minutes with the brake off. Remove the supernatant and resuspend the cells at  $1 \times 10^8$  cells/mL in recommended medium.

## Recommended Medium

EasySep™ Buffer (Catalog #20144), or PBS containing 2% FBS and 1 mM EDTA. Medium should be free of Ca++ and Mg++.

## Directions for Use – Manual EasySep™ Protocols

See page 1 for Sample Preparation and Recommended Medium. Refer to Tables 1 and 2 for detailed instructions regarding the EasySep™ procedure for each magnet.

**Table 1. EasySep™ Release Human CD3 Positive Selection Kit Protocol**

|      |                                                                                                                                                                                                               | EASYSEP™ MAGNETS                                                                                                          |                                                                                                                                                  |
|------|---------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|---------------------------------------------------------------------------------------------------------------------------|--------------------------------------------------------------------------------------------------------------------------------------------------|
| STEP | INSTRUCTIONS                                                                                                                                                                                                  |  <b>EasySep™</b><br>(Catalog #18000)<br> |  <b>“The Big Easy”</b><br>(Catalog #18001)<br>                |
| 1    | Prepare sample at the indicated cell concentration within the volume range.                                                                                                                                   | 1 x 10 <sup>8</sup> cells/mL<br>0.25 - 2 mL                                                                               | 1 x 10 <sup>8</sup> cells/mL<br>0.5 - 8 mL                                                                                                       |
|      | Add sample to required tube.                                                                                                                                                                                  | 5 mL (12 x 75 mm) polystyrene round-bottom tube<br>(e.g. Corning Catalog #352058)                                         | 14 mL (17 x 100 mm) polystyrene round-bottom tube<br>(e.g. Corning Catalog #352057)                                                              |
| 2    | Add Selection Cocktail to sample.                                                                                                                                                                             | 100 µL/mL of sample                                                                                                       | 100 µL/mL of sample                                                                                                                              |
|      | Mix and incubate.                                                                                                                                                                                             | RT for 3 minutes                                                                                                          | RT for 3 minutes                                                                                                                                 |
| 3    | Vortex Releasable RapidSpheres™.<br>NOTE: Particles should appear evenly dispersed.                                                                                                                           | 30 seconds                                                                                                                | 30 seconds                                                                                                                                       |
| 4    | Add Releasable RapidSpheres™ to sample.                                                                                                                                                                       | 100 µL/mL of sample                                                                                                       | 100 µL/mL of sample                                                                                                                              |
|      | Mix and incubate.                                                                                                                                                                                             | RT for 3 minutes                                                                                                          | RT for 3 minutes                                                                                                                                 |
| 5    | Add recommended medium to top up the sample to the indicated volume. Mix by gently pipetting up and down 2 - 3 times.                                                                                         | Top up to 2.5 mL                                                                                                          | <ul style="list-style-type: none"> <li>• Top up to 5 mL for samples ≤ 3 mL</li> <li>• Top up to 10 mL for samples &gt; 3 mL</li> </ul>           |
|      | Place the tube (without lid) into the magnet and incubate.                                                                                                                                                    | RT for 5 minutes                                                                                                          | RT for 5 minutes                                                                                                                                 |
| 6    | Pick up the magnet, and in one continuous motion invert the magnet and tube,* pouring off the supernatant. Remove the tube from the magnet; this tube contains the isolated cells.                            | Discard supernatant                                                                                                       | Discard supernatant                                                                                                                              |
| 7    | Repeat steps as indicated.                                                                                                                                                                                    | Steps 5 and 6, two more times<br>(total of 3 x 5-minute separations)                                                      | Steps 5 and 6, two more times<br>(total of 3 x 5-minute separations)                                                                             |
| 8    | Remove the tube from the magnet and add recommended medium to top up the sample to the indicated volume. Mix by gently pipetting up and down 2 - 3 times. Be sure to collect cells off the sides of the tube. | Same volume as the original starting sample volume<br>(i.e. same volume used in step 1)                                   | Same volume as the original starting sample volume<br>(i.e. same volume used in step 1)                                                          |
| 9    | Add Release Buffer to sample.                                                                                                                                                                                 | 100 µL/mL of sample                                                                                                       | 100 µL/mL of sample                                                                                                                              |
|      | Mix and incubate.                                                                                                                                                                                             | RT for 3 minutes                                                                                                          | RT for 3 minutes                                                                                                                                 |
| 10   | Add recommended medium to top up the sample to the indicated volume. Mix by gently pipetting up and down 2 - 3 times.                                                                                         | Top up to 2.5 mL                                                                                                          | <ul style="list-style-type: none"> <li>• Top up to 5 mL for start sample ≤ 3 mL</li> <li>• Top up to 10 mL for start sample &gt; 3 mL</li> </ul> |
|      | Place the tube (without lid) into the magnet and incubate.                                                                                                                                                    | RT for 5 minutes                                                                                                          | RT for 5 minutes                                                                                                                                 |
| 11   | Pick up the magnet, and in one continuous motion invert the magnet and tube,* pouring the enriched cell suspension into a new tube.                                                                           | Isolated cells (in the new tube) are ready for use                                                                        | Isolated cells (in the new tube) are ready for use                                                                                               |

RT - room temperature (15 - 25°C)

\* Leave the magnet and tube inverted for 2 - 3 seconds, then return upright. Do not shake or blot off any drops that may remain hanging from the mouth of the tube.

**Table 2. EasySep™ Release Human CD3 Positive Selection Kit Protocol**

| STEP | INSTRUCTIONS                                                                                                                                                                                                           | EASYSEP™ MAGNETS                                                                                                        |                                                                                                                                             |                                                                                                                                        |
|------|------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|-------------------------------------------------------------------------------------------------------------------------|---------------------------------------------------------------------------------------------------------------------------------------------|----------------------------------------------------------------------------------------------------------------------------------------|
|      |                                                                                                                                                                                                                        |  <b>EasyPlate™</b><br>(Catalog #18102) |  <b>EasyEights™</b> (Catalog #18103)<br><b>5 mL tube</b> |  <b>14 mL tube</b>                                  |
| 1    | Prepare sample at the indicated cell concentration within the volume range.                                                                                                                                            | 1 x 10 <sup>8</sup> cells/mL<br>0.05 - 0.2 mL                                                                           | 1 x 10 <sup>8</sup> cells/mL<br>0.25 - 2 mL                                                                                                 | 1 x 10 <sup>8</sup> cells/mL<br>0.5 - 8 mL                                                                                             |
|      | Add sample to required tube (or plate when using the EasyPlate™ EasySep™ Magnet).                                                                                                                                      | Round-bottom, non-tissue culture-treated 96-well plate<br>(e.g. Corning Catalog #3788)                                  | 5 mL (12 x 75 mm) polystyrene round-bottom tube<br>(e.g. Corning Catalog #352058)                                                           | 14 mL (17 x 100 mm) polystyrene round-bottom tube<br>(e.g. Corning Catalog #352057)                                                    |
| 2    | Add Selection Cocktail to sample.                                                                                                                                                                                      | 100 µL/mL of sample                                                                                                     | 100 µL/mL of sample                                                                                                                         | 100 µL/mL of sample                                                                                                                    |
|      | Mix and incubate.                                                                                                                                                                                                      | RT for 3 minutes                                                                                                        | RT for 3 minutes                                                                                                                            | RT for 3 minutes                                                                                                                       |
| 3    | Vortex Releasable RapidSpheres™.<br>NOTE: Particles should appear evenly dispersed.                                                                                                                                    | 30 seconds                                                                                                              | 30 seconds                                                                                                                                  | 30 seconds                                                                                                                             |
| 4    | Add Releasable RapidSpheres™ to sample.                                                                                                                                                                                | 100 µL/mL of sample                                                                                                     | 100 µL/mL of sample                                                                                                                         | 100 µL/mL of sample                                                                                                                    |
|      | Mix and incubate.                                                                                                                                                                                                      | RT for 3 minutes                                                                                                        | RT for 3 minutes                                                                                                                            | RT for 3 minutes                                                                                                                       |
| 5    | Add recommended medium to top up sample to the indicated volume. Mix by gently pipetting up and down 2 - 3 times.                                                                                                      | Top up to 0.25 mL                                                                                                       | Top up to 2.5 mL                                                                                                                            | <ul style="list-style-type: none"> <li>• Top up to 5 mL for samples ≤ 3 mL</li> <li>• Top up to 10 mL for samples &gt; 3 mL</li> </ul> |
|      | Place the tube or plate (without lid) into the magnet and incubate.                                                                                                                                                    | RT for 5 minutes                                                                                                        | RT for 5 minutes                                                                                                                            | RT for 5 minutes                                                                                                                       |
| 6    | Carefully pipette** (do not pour) off the supernatant. Remove the tube or plate, containing the isolated cells, from the magnet.                                                                                       | Discard supernatant                                                                                                     | Discard supernatant                                                                                                                         | Discard supernatant                                                                                                                    |
| 7    | Repeat steps as indicated.                                                                                                                                                                                             | Steps 5 and 6, two more times<br>(total of 3 x 5-minute separations)                                                    | Steps 5 and 6, two more times<br>(total of 3 x 5-minute separations)                                                                        | Steps 5 and 6, two more times<br>(total of 3 x 5-minute separations)                                                                   |
| 8    | Remove the tube or plate from the magnet and add recommended medium to top up the sample to the indicated volume. Mix by gently pipetting up and down 2 - 3 times. Be sure to collect cells off the sides of the tube. | Same volume as the original starting sample volume<br>(i.e. same volume used in step 1)                                 | Same volume as the original starting sample volume<br>(i.e. same volume used in step 1)                                                     | Same volume as the original starting sample volume<br>(i.e. same volume used in step 1)                                                |
| 9    | Add Release Buffer to sample.                                                                                                                                                                                          | 100 µL/mL of sample                                                                                                     | 100 µL/mL of sample                                                                                                                         | 100 µL/mL of sample                                                                                                                    |
|      | Mix and incubate.                                                                                                                                                                                                      | RT for 3 minutes                                                                                                        | RT for 3 minutes                                                                                                                            | RT for 3 minutes                                                                                                                       |
| 10   | Add recommended medium to top up the sample to the indicated volume. Mix by gently pipetting up and down 2 - 3 times.                                                                                                  | Top up to 0.25 mL                                                                                                       | Top up to 2.5 mL                                                                                                                            | <ul style="list-style-type: none"> <li>• Top up to 5 mL for samples ≤ 3 mL</li> <li>• Top up to 10 mL for samples &gt; 3 mL</li> </ul> |
|      | Place the tube or plate (without lid) into the magnet and incubate.                                                                                                                                                    | RT for 5 minutes                                                                                                        | RT for 5 minutes                                                                                                                            | RT for 5 minutes                                                                                                                       |
| 11   | Carefully pipette** (do not pour) off the enriched cell suspension into a new tube.                                                                                                                                    | Isolated cells (in the new tube) are ready for use                                                                      | Isolated cells (in the new tube) are ready for use                                                                                          | Isolated cells (in the new tube) are ready for use                                                                                     |

RT - room temperature (15 - 25°C)

\*\* Collect the entire supernatant, all at once, into a single pipette (e.g. for the EasyEights™ 5 mL tube use a 2 mL serological pipette and for the EasyEights™ 14 mL tube use a 10 mL serological pipette).

## Notes and Tips

### ASSESSING PURITY

The EasySep™ Release Human CD3 Positive Selection Cocktail uses an anti-CD3 antibody clone that to our knowledge fully or partially blocks all anti-CD3 antibody clones used to assess purity by flow cytometry. For purity assessment of CD3+ cells by flow cytometry use the following fluorochrome-conjugated antibody clone:

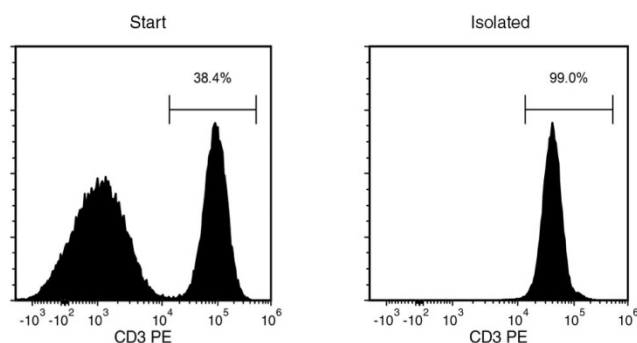
- Anti-Human CD3 Antibody, Clone UCHT1 (Catalog #60011; partially blocked)

NOTE: Brilliant Violet™ antibody conjugates should be carefully titrated on EasySep™ Release-isolated cells prior to analysis by flow cytometry or fluorescence microscopy. For recommended staining conditions, contact us at [techsupport@stemcell.com](mailto:techsupport@stemcell.com).

One of the following methods can also be used:

- Use alternative markers such as fluorochrome-conjugated Anti-Human CD4 Antibody, Clone OKT4 (Catalog #60016) and Anti-Human CD8a Antibody, Clone RPA-T8 (Catalog #60022).
- Use a fluorochrome-conjugated secondary antibody, such as Goat Anti-Mouse IgG (H+L) Antibody, Polyclonal (Catalog #60138).

## Data



Starting with a single-cell suspension of human PBMCs, the CD3+ cell content of the isolated fraction is typically  $98.7 \pm 0.9\%$  (mean  $\pm$  SD using the purple EasySep™ Magnet). In the above example, the purities of the start and final isolated fractions are 38.4% and 99.0%, respectively.

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