

# EasySep™ Human CD4+ T Cell Isolation Kit

Negative Selection  
Catalog #17952

For processing 1 x 10<sup>9</sup> cells



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## Description

Isolate untouched and highly purified CD4+ T cells from fresh or previously frozen human peripheral blood mononuclear cells (PBMCs) or washed leukapheresis samples in as little as 8 minutes by immunomagnetic negative selection.

- Fast, easy-to-use and column-free
- Up to 97% purity with high recovery
- Untouched, viable cells

This kit targets non-CD4+ T cells for removal with antibodies recognizing specific cell surface markers. Unwanted cells are labeled with antibodies and magnetic particles, and separated without columns using an EasySep™ magnet. Desired cells are simply poured off into a new tube. Isolated cells are immediately available for downstream applications such as flow cytometry, culture, or DNA/RNA extraction.

## Component Descriptions

| COMPONENT NAME                                | COMPONENT # | QUANTITY | STORAGE                             | SHELF LIFE                               | FORMAT   |
|---|-------------|----------|-------------------------------------|--|--|
| EasySep™ Human CD4+ T Cell Isolation Cocktail | 17952C      | 1 x 1 mL | Store at 2 - 8°C.<br>Do not freeze. | Stable until expiry date (EXP) on label. | A combination of monoclonal antibodies in PBS. |
| EasySep™ Dextran RapidSpheres™ 50103          | 50103       | 1 x 1 mL | Store at 2 - 8°C.<br>Do not freeze. | Stable until expiry date (EXP) on label. | A suspension of magnetic particles in water.   |

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 aggregated suspensions through a 40 µm Cell Strainer (Catalog #27305) for optimal results.

After preparation, resuspend cells at 5 x 10<sup>7</sup> 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 with 2% fetal bovine serum (FBS) and centrifuging at 500 x g for 10 minutes at room temperature (15 - 25°C). Remove the supernatant and resuspend the cells at 5 x 10<sup>7</sup> cells/mL in recommended medium.



## Recommended Medium

EasySep™ Buffer (Catalog #20144), RoboSep™ Buffer (Catalog #20104), or PBS containing 2% FBS and 1 mM EDTA. Medium should be free of Ca<sup>++</sup> and Mg<sup>++</sup>.

**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™ Human CD4+ T Cell Isolation Kit Protocol**

|      |   | EASYSEP™ MAGNETS  |  |
|------|---|---|--|
| STEP | INSTRUCTIONS  |  <b>EasySep™</b><br>(Catalog #18000) |  <b>“The Big Easy”</b><br>(Catalog #18001)          |
| 1    | Prepare sample at the indicated cell concentration within the volume range.   | 5 x 10 <sup>7</sup> cells/mL<br>0.25 - 2 mL   | 5 x 10 <sup>7</sup> cells/mL<br>1 - 8.5 mL   |
|      | Add sample to required tube.  | 5 mL (12 x 75 mm) polystyrene round-bottom tube (e.g. Catalog #38007)   | 14 mL (17 x 95 mm) polystyrene round-bottom tube (e.g. Catalog #38008)   |
| 2    | Add Isolation Cocktail to sample.   | 50 µL/mL of sample  | 50 µL/mL of sample   |
|      | Mix and incubate.   | RT for 5 minutes  | RT for 5 minutes   |
| 3    | Vortex RapidSpheres™.<br>NOTE: Particles should appear evenly dispersed.  | 30 seconds  | 30 seconds   |
| 4    | Add RapidSpheres™ to sample and mix.  | 50 µL/mL of sample  | 50 µL/mL of sample   |
| 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 ≤ 4 mL</li> <li>• Top up to 10 mL for samples &gt; 4 mL</li> </ul> |
|      | Place the tube (without lid) into the magnet and incubate.  | RT for 3 minutes  | RT for 3 minutes   |
| 6    | 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 are ready for use  | Isolated cells 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™ Human CD4+ T Cell Isolation Kit Protocol**

| STEP | INSTRUCTIONS   | EASYSEP™ MAGNETS  |   |  |   |
|------|--|---|---|--|---|
|      |  |  <b>EasyPlate™</b><br>(Catalog #18102) |  <b>EasyEights™ (Catalog #18103)</b> |  |  <b>Easy 50</b><br>(Catalog #18002)                    |
|      |  |   | 5 mL tube   | 14 mL tube   |   |
| 1    | Prepare sample at the indicated cell concentration within the volume range.  | 5 x 10 <sup>7</sup> cells/mL<br>0.1 - 0.2 mL  | 5 x 10 <sup>7</sup> cells/mL<br>0.5 - 2 mL  | 5 x 10 <sup>7</sup> cells/mL<br>1 - 8.5 mL   | 5 x 10 <sup>7</sup> cells/mL<br>10 - 45 mL  |
|      | Add sample to required tube (or plate when using the EasySep™ EasyPlate™ Magnet).  | Round-bottom, non-tissue culture-treated 96-well plate (e.g. Catalog #38018)  | 5 mL (12 x 75 mm) polystyrene round-bottom tube (e.g. Catalog #38007)   | 14 mL (17 x 95 mm) polystyrene round-bottom tube (e.g. Catalog #38008)   | 50 mL (30 x 115 mm) conical tube (e.g. Catalog #38010)  |
| 2    | Add Isolation Cocktail to sample.  | 50 µL/mL of sample  | 50 µL/mL of sample  | 50 µL/mL of sample   | 50 µL/mL of sample  |
|      | Mix and incubate.  | RT for 5 minutes  | RT for 5 minutes  | RT for 5 minutes   | RT for 5 minutes  |
| 3    | Vortex RapidSpheres™.<br>NOTE: Particles should appear evenly dispersed.   | 30 seconds  | 30 seconds  | 30 seconds   | 30 seconds  |
| 4    | Add RapidSpheres™ to sample and mix.   | 50 µL/mL of sample  | 50 µL/mL of sample  | 50 µL/mL of sample   | 50 µL/mL of sample  |
| 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 ≤ 4 mL</li> <li>• Top up to 10 mL for samples &gt; 4 mL</li> </ul> | <ul style="list-style-type: none"> <li>• Top up to 25 mL for samples ≤ 20 mL</li> <li>• Top up to 50 mL for samples &gt; 20 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   | RT for 10 minutes   |
| 6    | Carefully pipette** (do not pour) the enriched cell suspension into new tube or plate.   | Use a new well in the 96-well plate   | Use a new 5 mL tube   | Use a new 14 mL tube   | Use a new 50 mL tube  |
| 7    | Remove the tube or plate, containing the isolated cells, from the magnet and place the new tube or plate (without lid) into the magnet and incubate for a second separation. | RT for 5 minutes  | RT for 5 minutes  | RT for 5 minutes   | RT for 5 minutes  |
| 8    | Carefully pipette** (do not pour) the enriched cell suspension into new tube or plate.   | Isolated cells are ready for use  | Isolated cells are ready for use  | Isolated cells are ready for use   | Isolated cells are ready for use  |


RT - room temperature (15 - 25°C)

\*\* Collect the entire supernatant, all at once, into a single pipette (e.g. for EasyEights™ 5 mL tube use a 2 mL serological pipette [Catalog #38002]; for EasyEights™ 14 mL tube use a 10 mL serological pipette [Catalog #38004]).

## Directions for Use – Fully Automated RoboSep™ Protocol

See page 1 for Sample Preparation and Recommended Medium. Refer to Table 3 for detailed instructions regarding the RoboSep™ procedure.

**Table 3. RoboSep™ Human CD4+ T Cell Isolation Kit Protocol**

| STEP | INSTRUCTIONS   | RoboSep™<br>(Catalog #20000 and #21000)                                   |  |
|------|--|---|---|
| 1    | Prepare sample at the indicated cell concentration within the volume range.                  | 5 x 10 <sup>7</sup> cells/mL<br>0.5 - 8.5 mL                              |   |
|      | Add sample to required tube.   | 14 mL (17 x 95 mm) polystyrene round-bottom tube<br>(e.g. Catalog #38008) |   |
| 2    | Select protocol.   | Human CD4+ T Cell Isolation 17952   |   |
| 3    | Vortex RapidSpheres™.<br>NOTE: Particles should appear evenly dispersed.                     | 30 seconds  |   |
| 4    | Load the carousel.   | Follow on-screen prompts  |   |
|      | Start the protocol.  | Press the green "Run" button  |   |
| 5    | Unload the carousel when the run is complete. Remove the tube containing the isolated cells. | Isolated cells are ready for use  |   |

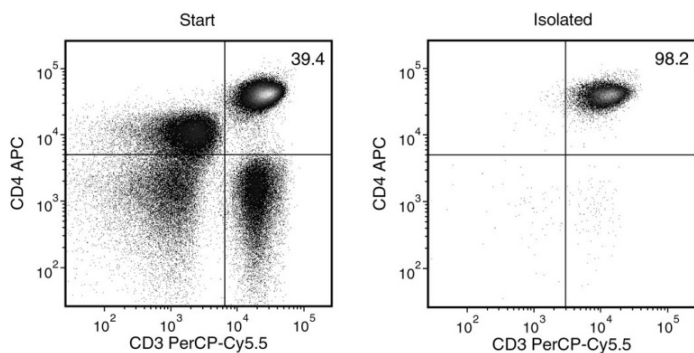
## Notes and Tips

### ASSESSING PURITY

For purity assessment of CD4+ T cells (CD3+CD4+) by flow cytometry, use the following fluorochrome-conjugated antibodies:

- Anti-Human CD3 Antibody, Clone UCHT1 (Catalog #60011), and
- Anti-Human CD4 Antibody, Clone OKT4 (Catalog #60016)

## Data



Starting with human PBMCs, the CD4+ T cell content (CD3+CD4+) of the isolated fraction is typically 94.8 ± 2.3% (mean ± SD using the purple EasySep™ Magnet). In the above example, the purities of the start and final isolated fractions are 39.4% and 98.2%, respectively.

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