



## EasySep™ Human Naïve CD4+ T Cell Isolation Kit II

Negative Selection

Catalog #17555

For processing  $1 \times 10^9$  cells



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## Description

Isolate untouched and highly purified naïve CD4+ T cells (CD3+CD4+CD45RA+CD45RO-) from fresh or previously frozen human peripheral blood mononuclear cells (PBMCs) in as little as 11 minutes by immunomagnetic negative selection.

- Fast, easy-to-use and column-free
- Up to 98% purity
- Untouched, viable cells

This kit targets non-naïve 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 Naïve CD4+ T Cell Isolation Cocktail II | 17555C      | 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. |
| EasySep™ Dextran RapidSpheres™ 50103                   | 50103       | 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.   |

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  $5 \times 10^7$  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)

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

1. Add an equal volume of Ammonium Chloride Solution to the leukapheresis sample.
2. Incubate on ice for 15 minutes.
3. Centrifuge at 500 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 with the brake off. Carefully remove the supernatant.
5. Repeat step 4 one or more times until most of the platelets have been removed (indicated by a clear supernatant).
6. Resuspend the cells at  $5 \times 10^7$  cells/mL in recommended medium.



## Recommended Medium

EasySep™ Buffer (Catalog #20144), RoboSep™ Buffer (Catalog #20104), or PBS containing 2% fetal bovine serum (FBS) with 1 mM EDTA. Medium should be free of Ca++, Mg++, and biotin.

## 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 Naïve CD4+ T Cell Isolation Kit II 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>0.1 - 2 mL  | 5 x 10 <sup>7</sup> cells/mL<br>0.25 - 8.5 mL  |
|      | Add sample to required tube.  | 5 mL (12 x 75 mm) polystyrene round-bottom tube<br>(e.g. Catalog #38007)  | 14 mL (17 x 95 mm) polystyrene round-bottom tube<br>(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<br>No incubation, IMMEDIATELY move to next step  | 50 µL/mL of sample<br>No incubation, IMMEDIATELY move to next step   |
| 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 &lt; 4 mL</li> <li>• Top up to 10 mL for samples ≥ 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. | Use a new 5 mL tube   | Use a new 14 mL tube   |
| 7    | Remove the tube from the magnet and place the new tube (without lid) into the magnet and incubate for a second separation.          | RT for 3 minutes  | RT for 3 minutes   |
| 8    | 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 Naïve CD4+ T Cell Isolation Kit II Protocol**

|      |  | EASYSEP™ MAGNETS  |  |   |
|------|--|---|--|---|
| STEP | INSTRUCTIONS   | EasyEights™ (Catalog #18103)  |  | Easy 50 ‡ (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>0.25 - 2 mL                                 | 5 x 10 <sup>7</sup> cells/mL<br>2 - 8.5 mL   | 5 x 10 <sup>7</sup> cells/mL<br>5 - 40 mL   |
|      | Add sample to required tube.   | 5 mL (12 x 75 mm)<br>polystyrene round-bottom tube<br>(e.g. Catalog #38007) | 14 mL (17 x 95 mm)<br>polystyrene round-bottom tube<br>(e.g. Catalog #38008)   | 50 mL (30 x 115 mm) conical tube<br>(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  |
|      | Mix and incubate.  | 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  |
| 4    | Add RapidSpheres™ to sample and mix.   | 50 µL/mL of sample<br>No incubation, IMMEDIATELY move to next step          | 50 µL/mL of sample<br>No incubation, IMMEDIATELY move to next step   | 100 µL/mL of sample<br>No incubation, IMMEDIATELY move to next step   |
| 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 2.5 mL  | <ul style="list-style-type: none"> <li>Top up to 5 mL for samples &lt; 4 mL</li> <li>Top up to 10 mL for samples ≥ 4 mL</li> </ul> | <ul style="list-style-type: none"> <li>Top up to 25 mL for samples ≤ 10 mL</li> <li>Top up to 50 mL for samples &gt; 10 mL</li> </ul> |
|      | Place the tube (without lid) into the magnet and incubate.   | RT for 5 minutes  | RT for 10 minutes  | RT for 10 minutes   |
| 6    | Carefully pipette** (do not pour) the enriched cell suspension into a new tube.  | Use a new 5 mL tube   | Use a new 14 mL tube   | Use a new 50 mL tube  |
| 7    | Remove the tube from the magnet and place the new tube (without lid) into the magnet and incubate for a second separation. | RT for 5 minutes  | RT for 10 minutes  | RT for 10 minutes   |
| 8    | Carefully pipette** (do not pour) the enriched cell suspension into a new tube. Collect only the clear fraction.           | Isolated cells are ready for use  | Isolated cells are ready for use   | Isolated cells are ready for use  |

RT - room temperature (15 - 25°C)


‡ When using the Easy 50 EasySep™ Magnet, contact us at [techsupport@stemcell.com](mailto:techsupport@stemcell.com) to request an additional vial of EasySep™ Dextran RapidSpheres™ 50103.

\*\* Collect the entire supernatant, all at once, into a single pipette (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 Naïve CD4+ T Cell Isolation Kit II 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>1 - 8.5 mL<br>NOTE: If starting with 2.5 - 5 x 10 <sup>7</sup> cells, resuspend cells in 1 mL. |   |
|      | Add sample to required tube.  | 14 mL (17 x 95 mm)<br>polystyrene round-bottom tube (e.g. Catalog #38008)  |   |
| 2    | Select protocol.  | Human Naïve CD4+ T Cell Isolation II 17555   |   |
| 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.                               | Isolated cells are ready for use   |   |

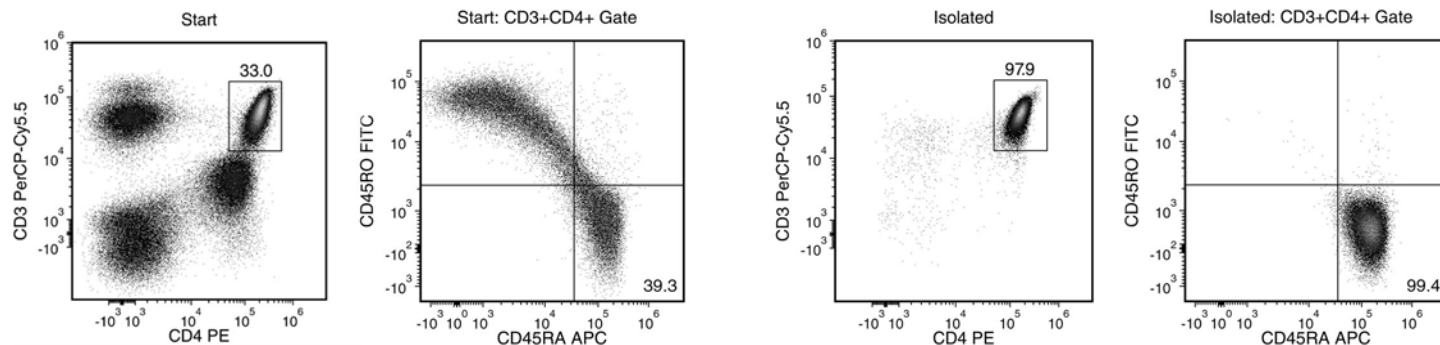
## Notes and Tips

### ASSESSING PURITY

For purity assessment of naïve CD4+ T cells (CD3+CD4+CD45RA+CD45RO-) by flow cytometry use the following fluorochrome-conjugated antibody clones:

- Anti-Human CD3 Antibody, Clone UCHT1 (Catalog #60011), and
- Anti-Human CD4 Antibody, Clone OKT4 (Catalog #60016), and
- Anti-Human CD45RO Antibody, Clone UCHL1 (Catalog #60097), and
- Anti-human CD45RA antibody

## Data



Starting with fresh mononuclear cells, the naïve CD4+ T cell content (CD3+CD4+CD45RA+CD45RO-) of the isolated fraction is typically 96.6 ± 1.5% (mean ± SD using the purple EasySep™ Magnet). In the above example, the purities of the start and final isolated fractions are 13.0% and 97.3%, respectively.

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