

EasySep™ Human T Cell Enrichment Kit

For processing 1×10^9 cells

Catalog #19051

Negative Selection

Document #1000000847 | Version 02



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Description

Isolate untouched and highly purified T cells from fresh or previously frozen human peripheral blood mononuclear cells (PBMCs) or lysed leukapheresis samples by immunomagnetic negative selection.

- Fast, easy-to-use and column-free
- Up to 99% purity
- Untouched, viable cells

This kit targets non-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 T Cell Enrichment Cocktail | 19051C.2 | 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™ D Magnetic Particles | 19250 | 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 TBS. |

PBS - phosphate-buffered saline; TBS - TRIS-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.

PERIPHERAL BLOOD

Prepare a PBMC suspension from whole peripheral 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 37 µm cell strainer (Catalog #27250) for optimal results.

After preparation, resuspend cells at 5×10^7 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

1. Add an equal volume of Ammonium Chloride Solution (Catalog #07800) to the Leukopak.

NOTE: If working with large volumes (> 150 mL), concentrate the Leukopak 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 and add 30 mL of Ammonium Chloride Solution). For small volumes (≤ 150 mL), add Ammonium Chloride Solution directly to the Leukopak.

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 5×10^7 cells/mL in recommended medium.



Recommended Medium

EasySep™ Buffer (Catalog #20144), RoboSep™ Buffer (Catalog #20104), or PBS containing 2% fetal bovine serum (FBS) 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™ Human T Cell Enrichment Kit Protocol

| | | EASYSEP™ MAGNETS | |
|------|-------------------------------------------------------------------------------------------------------------------------------------|-------------------------------------------------------------------------------------------------------------------|----------------------------------------------------------------------------------------------------------------------------------------|
| STEP | INSTRUCTIONS |  EasySep™ (Catalog #18000) |  “The Big Easy” (Catalog #18001) |
| 1 | Prepare sample at the indicated cell concentration within the volume range. | 5 x 10 ⁷ cells/mL 0.1 - 2 mL | 5 x 10 ⁷ cells/mL 0.25 - 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 Enrichment Cocktail to sample. | 50 µL/mL of sample | 50 µL/mL of sample |
| | Mix and incubate. | RT for 10 minutes | RT for 10 minutes |
| 3 | Vortex Magnetic Particles. NOTE: Particles should appear evenly dispersed. | 30 seconds | 30 seconds |
| 4 | Add Magnetic Particles to sample. | 50 µL/mL of sample | 50 µL/mL of sample |
| | Mix and incubate. | RT for 5 minutes | RT for 5 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"> • Top up to 5 mL for samples < 4 mL • Top up to 10 mL for samples ≥ 4 mL |
| | 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 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 T Cell Enrichment Kit Protocol

| STEP | INSTRUCTIONS | EASYSSEP™ MAGNETS | | | |
|------|-------------------------------------------------------------------------------------------------------------------|-------------------------------------------------------------------------------------------------------------------------|-------------------------------------------------------------------------------------------------------------------------|------------------------------------------------------------------------------------------------------------------------------------|---------------------------------------------------------------------------------------------------------------------------------------|
| | |  EasyPlate™ (Catalog #18102) |  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 ⁷ cells/mL 0.05 - 0.2 mL | 5 x 10 ⁷ cells/mL 0.25 - 2 mL | 5 x 10 ⁷ cells/mL 0.5 - 8 mL | 5 x 10 ⁷ cells/mL 1 - 40 mL |
| | Add sample to required tube (or plate when using the EasyPlate™ EasySep™ 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 Enrichment 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 10 minutes | RT for 10 minutes | RT for 10 minutes | RT for 10 minutes |
| 3 | Vortex Magnetic Particles. NOTE: Particles should appear evenly dispersed. | 30 seconds | 30 seconds | 30 seconds | 30 seconds |
| 4 | Add Magnetic Particles 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 10 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"> Top up to 5 mL for samples < 4 mL Top up to 10 mL for samples ≥ 4 mL | <ul style="list-style-type: none"> Top up to 25 mL for samples ≤ 10 mL Top up to 50 mL for samples > 10 mL |
| | Place the tube or plate (without lid) into the magnet and incubate. | RT for 10 minutes | RT for 5 minutes | RT for 5 minutes | RT for 10 minutes |
| 6 | Carefully pipette** (do not pour) the enriched cell suspension into a 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™ EasySep™ Human T Cell Enrichment Kit Protocol

| STEP | INSTRUCTIONS | RoboSep™ (Catalog #21000) |  |
|------|-------------------------------------------------------------------------------|---------------------------------------------------------------------------|-------------------------------------------------------------------------------------|
| 1 | Prepare sample at the indicated cell concentration within the volume range. | 5 x 10 ⁷ cells/mL 0.25 - 8 mL | |
| | Add sample to required tube. | 14 mL (17 x 95 mm) polystyrene round-bottom tube (e.g. Catalog #38008) | |
| 2 | Select protocol. | Human T Cell Negative Selection 19051 | |
| 3 | Vortex Magnetic Particles. 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 | |

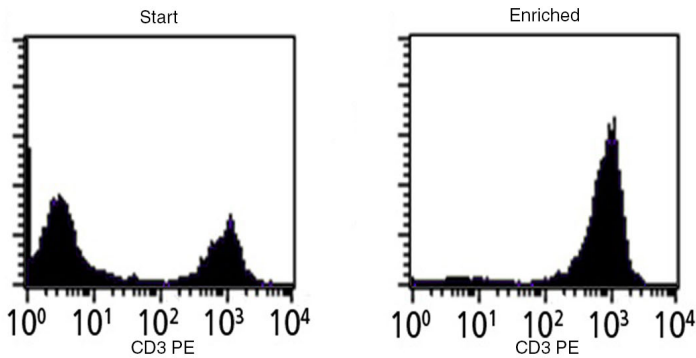
Notes and Tips

ASSESSING PURITY

For purity assessment of T cells by flow cytometry, use the following fluorochrome-conjugated antibody clones:

- Anti-Human CD3 Antibody, Clone UCHT1 (Catalog #60011), or
- Anti-Human CD4 Antibody, Clone OKT4 (Catalog #60016) and Anti-Human CD8a Antibody, Clone RPA-T8 (Catalog #60022)

Data



Starting with previously frozen mononuclear cells, the CD3+ cell content of the enriched fraction typically ranges from 95 - 99%. In the above example, the purities of the start and final enriched fractions are 35% and 97%, respectively.

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