



EasySep™ Direct Human T Cell Isolation Kit

Negative Selection

Catalog #19661

For processing 100 mL whole blood



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Description

Isolate highly purified T cells directly from human whole blood by immunomagnetic negative selection.

The benefits of this kit include:

- > 99.9% RBC depletion without the need for density gradient centrifugation, sedimentation or lysis
- Up to 97% purity of isolated cells
- Fast, easy-to-use and column-free
- Isolated cells are untouched

This kit targets non-T cells for removal with antibodies recognizing specific surface markers. Unwanted cells are labeled with antibodies and EasySep™ Direct RapidSpheres™, and separated using an EasySep™ magnet. Desired cells are simply collected into a new tube and 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™ Direct Human T Cell Isolation Cocktail | 19661C | 2 x 2.5 mL | Store at 2 - 8°C. | Stable until expiry date (EXP) on label. | A combination of monoclonal antibodies in PBS. |
| EasySep™ Direct RapidSpheres™ 50300 | 50300 | 4 x 2.5 mL | Store at 2 - 8°C. | Stable until expiry date (EXP) on label. | A suspension of magnetic particles and monoclonal antibodies in PBS. |

PBS - phosphate-buffered saline

Components may be shipped at room temperature (15 - 25°C) and should be refrigerated upon receipt.

Precipitate may be observed in the cocktail vial but will not affect performance.

Sample Preparation

For optimal RBC depletion, collect blood using heparin or acid citrate dextrose (ACD) as an anticoagulant. The use of K2EDTA or K3EDTA as an anticoagulant is not recommended.

For best recovery, use unprocessed human whole blood. Recovery of the desired isolated cells decreases with samples that are older than 24 hours.

The volume of blood that can be processed depends on the EasySep™ magnet used for the isolation procedure. Blood samples must be placed in the required tube to properly fit into the appropriate EasySep™ magnet (see Tables 1 and 2).

Recommended Medium

PBS (Catalog #37350) that is 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™ Direct Human T Cell Isolation Kit Protocol

| | | EASYSEP™ MAGNETS | |
|------|---|---|---|
| STEP | INSTRUCTIONS | EasySep™ (Catalog #18000) | “The Big Easy” (Catalog #18001) |
| 1 | Collect sample within the volume range. | 0.5 - 1.5 mL | 1.5 - 7.0 mL |
| | Add whole blood sample to required tube. | 5 mL (12 x 75 mm) polystyrene round-bottom tube (e.g. Corning Catalog #352058) | 14 mL (17 x 100 mm) polystyrene round-bottom tube (e.g. Corning Catalog #352057) |
| 2 | Vortex RapidSpheres™. | 30 seconds | 30 seconds |
| 3 | Add Isolation Cocktail to sample. | 50 µL/mL of sample | 50 µL/mL of sample |
| 4 | Add RapidSpheres™ to sample. | 50 µL/mL of sample | 50 µL/mL of sample |
| 5 | Mix and incubate. | RT for 5 minutes | RT for 5 minutes |
| 6 | 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 double the volume for samples ≤ 5 mL Top up to 10 mL for samples > 5 mL |
| 7 | Place the tube (without lid) into the magnet and incubate. | RT for 5 minutes | RT for 5 minutes |
| 8 | Pick up the magnet, and in one continuous motion invert the magnet and tube, pouring off the enriched cell suspension* into a new tube. | Use a new 5 mL tube | Use a new 14 mL tube |
| 9 | Add RapidSpheres™ to the new tube containing the enriched cells. | Use same volume as in step 4 | Use same volume as in step 4 |
| | Mix and incubate. | RT for 5 minutes | RT for 5 minutes |
| 10 | Remove the tube from the magnet and place the tube from step 9 (without lid) into the magnet and incubate for a second separation. | 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 are now ready for use | Use a new 14 mL tube |
| 12 | Remove the tube from the magnet and place the new tube (without lid) into the magnet and incubate for a third separation. | --- | RT for 5 minutes |
| 13 | 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 now ready for use |

RT; room temperature (15 - 25°C)

* Following the first magnetic separation the collected cells may contain a significant amount of RBCs and may look similar to the original unprocessed human whole blood sample.

** To minimize RBC contamination in the isolated cells, pour off the sample along a clean area of the tube (i.e. the opposite side to where the sample was poured in).

Table 2. EasySep™ Direct Human T Cell Isolation Kit Protocol

| STEP | INSTRUCTIONS | EASYSEP™ MAGNETS | | | Easy 50 (Catalog #18002) |
|------|--|------------------------------|--|---|---|
| | | EasyEights™ (Catalog #18103) | 5 mL tube | 14 mL tube | |
| 1 | Collect sample within the volume range. | | 0.5 - 1.5 mL | 1.5 - 7.0 mL | 7 - 30 mL |
| | Add whole blood sample to required tube. | | 5 mL (12 x 75 mm) polystyrene round-bottom tube (e.g. Corning Catalog #352058) | 14 mL (17 x 100 mm) polystyrene round-bottom tube (e.g. Corning Catalog #352057) | 50 mL conical tube (e.g. Corning Catalog #352070) |
| 2 | Vortex RapidSpheres™. | | 30 seconds | 30 seconds | 30 seconds |
| 3 | Add Isolation Cocktail to sample. | | 50 µL/mL of sample | 50 µL/mL of sample | 50 µL/mL of sample |
| 4 | Add RapidSpheres™ to sample. | | 50 µL/mL of sample | 50 µL/mL of sample | 50 µL/mL of sample |
| 5 | Mix and incubate. | | RT for 5 minutes | RT for 5 minutes | RT for 5 minutes |
| 6 | 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 double the volume for samples ≤ 5 mL Top up to 10 mL for samples > 5 mL | <ul style="list-style-type: none"> Top up to double the volume for samples ≤ 25 mL Top up to 50 mL for samples > 25 mL |
| 7 | Place the tube (without lid) into the magnet and incubate. | | RT for 5 minutes | RT for 5 minutes | RT for 10 minutes |
| 8 | Carefully pipette*** (do not pour) the enriched cell suspension into a new tube. NOTE: Collect the entire clear fraction from top to bottom. For optimal recovery, also collect a small volume of RBCs (up to 10% of the starting sample volume). | | Use a new 5 mL tube | Use a new 14 mL tube | Use a new 50 mL tube |
| 9 | Add RapidSpheres™ to the new tube containing the enriched cells. | | Use same volume as in step 4 | Use same volume as in step 4 | Use same volume as in step 4 |
| | Mix and incubate. | | RT for 5 minutes | RT for 5 minutes | RT for 5 minutes |
| 10 | Remove the tube from the magnet and place the tube from step 9 (without lid) into the magnet and incubate for a second round of separation. | | RT for 5 minutes | RT for 5 minutes | RT for 5 minutes |
| 11 | Carefully pipette*** (do not pour) the enriched cell suspension into a new tube. NOTE: Collect only the clear fraction. | | Use a new 5 mL tube | Use a new 14 mL tube | Use a new 50 mL tube |
| 12 | Remove the tube from the magnet and place the new tube (without lid) containing the enriched cells into the magnet and incubate for a third round of separation. | | RT for 5 minutes | RT for 5 minutes | RT for 5 minutes |
| 13 | Carefully pipette*** (do not pour) the enriched cell suspension into a new tube. NOTE: Collect only the clear fraction. | | Isolated cells are now ready for use | Isolated cells are now ready for use | Isolated cells are now ready for use |

RT; room temperature (15 - 25°C)

*** Collect the entire enriched cell suspension, 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

REMOVAL OF RESIDUAL RBCs IN THE ISOLATED CELLS

Typically, further RBC depletion is not required following cell isolation. If residual RBCs are visible in the isolated cell pellet following centrifugation after the end of the protocol, resuspend in a small volume (0.2 - 2.5 mL) of recommended medium or desired culture medium and place in a smaller EasySep™ magnet for an additional 5 minute separation. Collect the supernatant and the isolated cells are now ready for use in downstream applications. Residual RBCs may also be lysed using Ammonium Chloride Solution (Catalog #07800).

ASSESSING PURITY

For purity assessment of T cells by flow cytometry use fluorochrome-conjugated:

- Anti-Human CD3 Antibody, Clone UCHT1 (Catalog #60011), and
- Anti-Human CD45 Antibody, Clone HI30 (Catalog #60018)

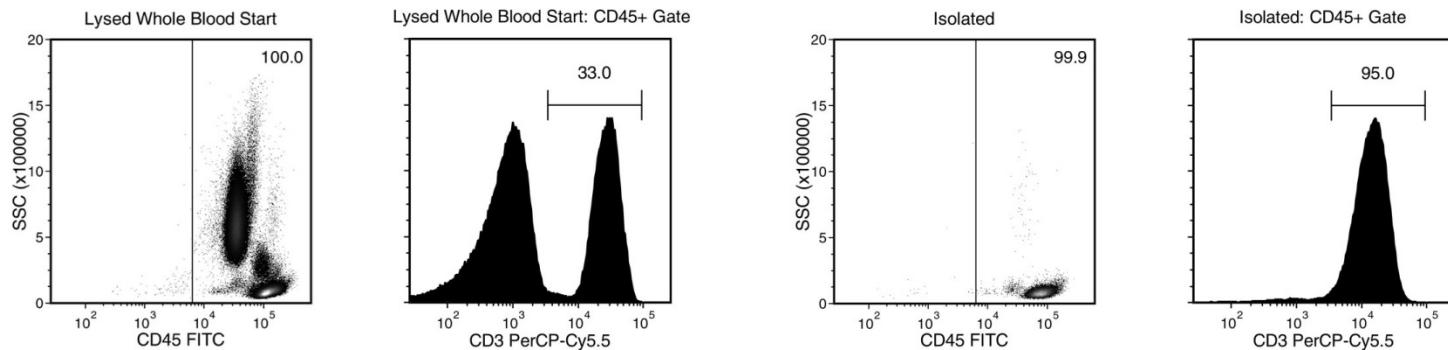
Purity may also be assessed using alternative fluorochrome-conjugated markers such as:

- Anti-Human CD4 Antibody, Clone OKT4 (Catalog #60016), and
- Anti-Human CD8 Antibody, Clone RPA-T4 (Catalog #60022), and
- Anti-Human CD45 Antibody, Clone HI30

NOTE: It is recommended to assess purity on the CD45 positive cells to exclude debris, platelets and RBCs.

Data

Starting with human whole blood from normal healthy donors, the typical T cell (CD3+) content of the non-lysed final isolated fraction is $95.3 \pm 1.4\%$ (gated on CD45) or $94.9 \pm 1.5\%$ (not gated on CD45).



In the above example, the T cell (CD3+) content of the lysed whole blood start sample and non-lysed final isolated fraction is 33.0% and 95.0% (gated on CD45), respectively, or 33.0% and 94.9% (not gated on CD45), respectively. The starting frequency of T cells in the non-lysed whole blood start sample above is 0.059% (data not shown).

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