# RoboSep™-C Human T Cell Isolation Kit

**For processing 1 x 10^10 cells**

Catalog #100-0204

Negative Selection

Document #10000008392 | Version 03

STEMCELL™ TECHNOLOGIES

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## Description

Isolate untouched and highly purified T cells from fresh human peripheral blood leukopaks using RoboSep™-C (Catalog #100-0185/100-0186) for automated immunomagnetic negative selection.

- Fully automated large-scale isolation in a closed system
- Up to 99% purity with high recovery
- Untouched, viable cells

This kit targets non-T cells for removal with antibodies recognizing specific cell surface markers. Using RoboSep™-C in a fully automated protocol, unwanted cells are labeled with antibodies and magnetic particles, and separated without columns using a RoboSep™-C magnet. Desired cells are washed and concentrated by RoboSep™-C and transferred to a final product bag. Isolated cells are immediately available for downstream applications such as flow cytometry, culture, or cryopreservation.

## Component Descriptions

| COMPONENT NAME | COMPONENT # | QUANTITY | STORAGE | SHELF LIFE | FORMAT |
|---|---|---|---|---|---|
| RoboSep™-C Human T Cell Isolation Cocktail | 300-0109 | 1 x 10 mL | Store at 2 - 8°C. Do not freeze. | Stable until expiry date (EXP) on label. | A combination of monoclonal antibodies in PBS. |
| RoboSep™-C Dextran RapidSpheres™ 30106 | 300-0106 | 1 x 10 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.

## Materials Required But Not Included

| PRODUCT NAME | CATALOG # |
|---|---|
| RoboSep™-C | 100-0185 OR 100-0186 |
| RoboSep™-C Tubing Set | 200-0090 |
| RoboSep™-C PBS/EDTA Buffer | 100-0188 |
| Fetal bovine serum (FBS) | e.g. 100-0179 |
| Tube sealer | e.g. Sebra 2600-1105 |
| 20 mm vial access devices | e.g. Yukon Medical YM-038 |
| Luer lock syringes | e.g. BD 309604/309653 |

## Sample Preparation

For available fresh human peripheral blood leukopaks, see www.stemcell.com/primarycells (e.g. Catalog #70500).

1. Determine the number of nucleated cells in the leukopak. Ensure the number of cells is between 2.5 and 20 x 10^9 cells. For > 20 x 10^9 cells, split the sample into multiple runs.

   NOTE: Each RoboSep™-C Human T Cell Isolation Kit processes up to 10 x 10^9 cells. For leukopaks containing > 10 x 10^9 cells, multiple kits are required.

2. Using aseptic technique, transfer sample into Sample Bag 1 provided in the RoboSep™-C Tubing Set, as follows:
   a. Spike the line of Sample Bag 1 containing the in-line blood filter into a spike port of the leukopak bag.
   b. Drain the contents of the leukopak bag into Sample Bag 1 by gravity. Flick the blood filter to minimize retention of sample volume in the line.
   c. Close the pinch clamp on the Sample Bag 1 line and use a tube sealer to seal off the tubing at a site between the blood filter and the sample bag. Remove and discard the sealed-off tubing.
3. Follow on-screen prompts to load Sample Bag 1 onto the instrument (Table 1).

## Recommended Medium

Use aseptic technique to prepare recommended medium (RoboSep™-C PBS/EDTA Buffer + 2% FBS), as follows:

1. Using a syringe, inject 60 mL of FBS into the RoboSep™-C PBS/EDTA Buffer bag via the luer injection port on the bag.
2. Invert bag several times to mix thoroughly.

   NOTE: If not used immediately, store recommended medium at 2 - 8°C. Do not exceed the shelf life of the individual components. Warm to room temperature (15 - 25°C) before use.

## Directions for Use – Fully Automated RoboSep™ Protocol

See pages 1 and 2 for Sample Preparation and Recommended Medium. Refer to Table 1 for detailed instructions regarding the RoboSep™-C procedure.

For RoboSep™-C setup instructions, refer to the RoboSep™-C User Reference Manual (Document #10000008334), available at www.stemcell.com or contact us to request a copy. Use aseptic technique for all steps.

**Table 1. RoboSep™-C Human T Cell Isolation Kit Protocol**

| STEP | INSTRUCTIONS | RoboSep™-C (Catalog #100-0185/100-0186) |
|---|---|---|
| 1 | **Select kit.** | Human T Cell Isolation 100-0204 |
| 2 | **Select protocol corresponding to number of cells in sample.** | • 2.5 to < 5 x 10^9 cells<br>• 5 to < 10 x 10^9 cells<br>• 10 to 20 x 10^9 cells |
| 3 | **Install Tubing Set.** | Follow on-screen prompts. |
| 4 | **Connect Recommended Medium:**<br>• **Remove cap from the primary buffer spike on the Tubing Set and connect it to the Recommended Medium Bag.**<br>• **Squeeze bag to prime filter.**<br>• **Hang Recommended Medium Bag on Weight Scale 7.** | Follow on-screen prompts. |
| 5 | **Add Isolation Cocktail to Process Bag 3:**<br>• **Attach a 20 mm vial access device to the cocktail vial.**<br>• **Connect a syringe to the vial access device, invert the vial, and draw up the indicated volume of cocktail plus a 2 mL bolus of air. Disconnect the syringe from the vial.**<br>• **Remove Process Bag 3 from Weight Scale 3.**<br>• **Remove cap from the injection port on Process Bag 3.**<br>• **With the injection port pointing up, inject the Isolation Cocktail followed by the bolus of air into Process Bag 3.**<br>• **Invert bag to mix and re-hang on Weight Scale 3.** | Use 1 mL cocktail per 10^9 cells<br>(e.g. for 10 x 10^9 cells, inject 10 mL of cocktail). |
| 6 | **Add Dextran RapidSpheres™ to Process Bag 2:**<br>• **Vortex RapidSpheres™ for 30 seconds. Particles should appear evenly dispersed.**<br>• **Attach a 20 mm vial access device to the particle vial.**<br>• **Connect a syringe to the vial access device, invert the vial, and draw up the indicated volume of particles plus a 2 mL bolus of air. Disconnect the syringe from the vial.**<br>• **Remove Process Bag 2 from Weight Scale 2.**<br>• **Remove cap from the injection port on Process Bag 2.**<br>• **With the injection port pointing up, inject the particles followed by the bolus of air into Process Bag 2.**<br>• **Invert bag to mix and re-hang on Weight Scale 2.** | Use 0.8 mL particles per 10^9 cells<br>(e.g. for 10 x 10^9 cells, inject 8 mL of particles). |
| 7 | **Connect Sample Bag 1:**<br>• **Remove cap from a spike port on Sample Bag 1.**<br>• **Remove cap from the Start Sample Spike and connect it to Sample Bag 1.**<br>• **Hang Sample Bag 1 on Weight Scale 1.** | Follow on-screen prompts. |
| 8 | **Collect isolated cells when the run is complete:**<br>• **Close pinch clamp below Process Bag 4.**<br>• **Seal the tubing below pinch clamp using a tube sealer and remove Process Bag 4 containing the isolated cells.** | Isolated cells are in Process Bag 4 on Weight Scale 4. |

## Notes and Tips

TROUBLESHOOTING

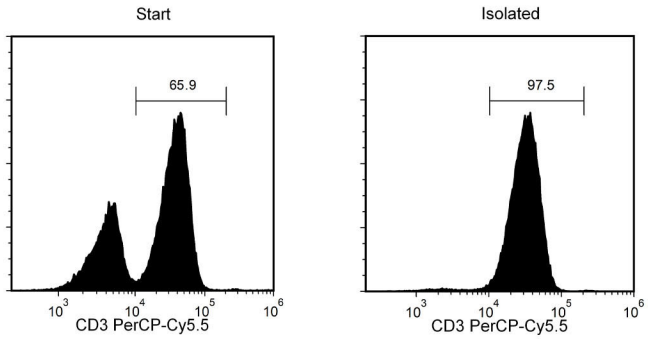
For troubleshooting tips, refer to the RoboSep™-C User Reference Manual (Document #10000008334), available at www.stemcell.com or contact us to request a copy.

ASSESSING PURITY

For purity assessment of CD3+ 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 a fresh human peripheral blood leukopak, the T cell content (CD3+) of the isolated fraction is typically 96.0 ± 3.2% (mean ± SD). In the above example, the purities of the start and final isolated fractions are 65.9% and 97.5%, respectively.