RoboSep™-C Human CD8+ T Cell Isolation Kit

For processing 1 x 10^10 cells

Catalog #100-0202

Negative Selection

Document #10000008422 | Version 03

STEMCELL™ TECHNOLOGIES

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Description

Isolate untouched and highly purified CD8+ 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 94% purity with high recovery
- Untouched, viable cells

This kit targets non-CD8+ 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 CD8+ T Cell Isolation Cocktail	300-0108	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 CD8+ 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 CD8+ T Cell Isolation Kit Protocol

STEP	INSTRUCTIONS	RoboSep™-C (Catalog #100-0185/100-0186)
1	**Select kit.**	Human CD8+ T Cell Isolation 100-0202
2	**Select protocol corresponding to number of cells in sample.**	• 2.5 to < 5 x 10^9 cells* • 5 to < 10 x 10^9 cells • 10 to 20 x 10^9 cells
3	**Install Tubing Set.**	Follow on-screen prompts.
4	**Connect Recommended Medium:** • **Remove cap from the primary buffer spike on the Tubing Set and connect it to the Recommended Medium Bag.** • **Squeeze bag to prime filter.** • **Hang Recommended Medium Bag on Weight Scale 7.**	Follow on-screen prompts.
5	**Add Isolation Cocktail to Process Bag 3:** • **Attach a 20 mm vial access device to the cocktail vial.** • **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.** • **Remove Process Bag 3 from Weight Scale 3.** • **Remove cap from the injection port on Process Bag 3.** • **With the injection port pointing up, inject the Isolation Cocktail followed by the bolus of air into Process Bag 3.** • **Invert bag to mix and re-hang on Weight Scale 3.**	Use 1 mL cocktail per 10^9 cells (e.g. for 10 x 10^9 cells, inject 10 mL of cocktail).
6	**Add Dextran RapidSpheres™ to Process Bag 2:** • **Vortex RapidSpheres™ for 30 seconds. Particles should appear evenly dispersed.** • **Attach a 20 mm vial access device to the particle vial.** • **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.** • **Remove Process Bag 2 from Weight Scale 2.** • **Remove cap from the injection port on Process Bag 2.** • **With the injection port pointing up, inject the particles followed by the bolus of air into Process Bag 2.** • **Invert bag to mix and re-hang on Weight Scale 2.**	Use 1 mL particles per 10^9 cells (e.g. for 10 x 10^9 cells, inject 10 mL of particles).
7	**Connect Sample Bag 1:** • **Remove cap from a spike port on Sample Bag 1.** • **Remove cap from the Start Sample Spike and connect it to Sample Bag 1.** • **Hang Sample Bag 1 on Weight Scale 1.**	Follow on-screen prompts.
8	**Collect isolated cells when the run is complete:** • **Close pinch clamp below Process Bag 4.** • **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.

* The kit is optimized for starting samples between 5 and 20 x 10^9 cells. Performance may vary when starting with samples between 2.5 and < 5 x 10^9 cells.

Notes and Tips

TROUBLESHOOTING

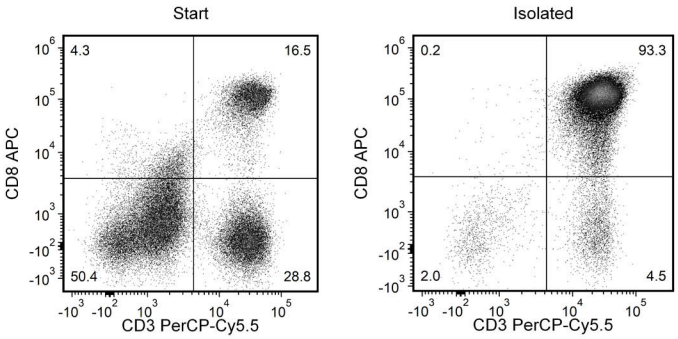
For troubleshooting tips, refer to the RoboSep™-C User Reference Manual (Document #10000008334), available at www.stemcell.com or contact us to request a copy.

ASSESSING PURITY

For purity assessment of CD8+ T cells (CD3+CD8+) by flow cytometry, use the following fluorochrome-conjugated antibody clones:

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

Data



Starting with a fresh human peripheral blood leukopak, the CD8+ T cell content (CD3+CD8+) of the isolated fraction is typically 86.8 ± 5.4% (mean ± SD). In the above example, the purities of the start and final isolated fractions are 16.5% and 93.3%, respectively.

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