EasySep™ Human CD8 Positive Selection Kit II

For processing 1 x 10^10 cells using the Easy 250 EasySep™ Magnet

Catalog #100-0699

Positive Selection

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Description

Isolate highly purified CD8+ cells from leukapheresis samples by immunomagnetic positive selection.

- Fast and easy-to-use
- Up to 99% purity with high recovery
- No columns required

This kit targets CD8+ cells for positive selection with antibodies recognizing the CD8 surface marker. Desired cells are labeled with antibodies and magnetic particles, and separated without columns using an EasySep™ magnet. Unwanted cells are simply pipetted off, while desired cells remain in the flask. Isolated cells are immediately available for downstream applications such as flow cytometry, culture, or DNA/RNA extraction.

NOTE: This is the Product Information Sheet (PIS) for isolating CD8+ cells using the Easy 250 EasySep™ Magnet (Catalog #100-0821). If using other magnets, refer to the applicable PIS, available at www.stemcell.com or contact us to request a copy.

Component Descriptions

COMPONENT NAME	COMPONENT #	QUANTITY	STORAGE	SHELF LIFE	FORMAT
EasySep™ Human CD8 Positive Selection Cocktail II	300-0363	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 with 10% HPCD and 0.09% rHA. Includes an Fc receptor blocking antibody.
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.

HPCD - 2-hydroxypropyl-β-cyclodextrin; PBS - phosphate-buffered saline; rHA - recombinant human albumin

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.

LYSED 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 300 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 300 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 120 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 1 x 10^8 cells/mL in recommended medium.

NOTE: Working with lysed leukapheresis samples is recommended for optimal performance. Alternatively, washed leukapheresis samples may be used (see below) for faster sample processing, but a reduction in performance may be observed.

WASHED LEUKAPHERESIS

Wash the peripheral blood leukapheresis sample by adding an equivalent volume of recommended medium or PBS containing 2% fetal bovine serum (FBS). Centrifuge at 300 x g for 10 minutes at room temperature (15 - 25°C). If platelet removal is necessary, centrifuge at 120 x g for 10 minutes with the brake off. Remove the supernatant and resuspend the cells at 1 x 10^8 cells/mL in recommended medium.

Recommended Medium

EasySep™ Buffer (Catalog #20144), RoboSep™ Buffer (Catalog #20104), or PBS containing 2% 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 Table 1 for detailed instructions regarding the EasySep™ procedure.

Table 1. EasySep™ Human CD8 Positive Selection Kit II Protocol

STEP	INSTRUCTIONS	Easy 250 EasySep™ Magnet (Catalog #100-0821)
1	Prepare sample at the indicated cell concentration within the volume range.	1 x 10^8 cells/mL 40 - 125 mL
	Add sample to required flask.	T-75 cm^2 cell culture flask (i.e. Corning Catalog #353135)
2	Add Selection Cocktail to sample. NOTE: Do not vortex cocktail.	100 µL/mL of sample
	Mix and incubate.	RT for 3 minutes
3	Vortex RapidSpheres™. NOTE: Particles should appear evenly dispersed.	30 seconds
4	Add RapidSpheres™ to sample.	6 µL/mL of sample
	Mix and incubate.	RT for 3 minutes
5	Add recommended medium to top up sample to the indicated volume. Be sure to resuspend the cells from the side of the flask. Mix by gently pipetting up and down 2 - 3 times.	Top up to double the original sample volume
	Place the flask (without cap) into the magnet and incubate.	RT for 10 minutes
6	Carefully pipette (do not pour) off the supernatant. Remove the flask, containing the isolated cells, from the magnet.	Discard supernatant
7	Add recommended medium to top up sample to the indicated volume. Be sure to resuspend the cells from the side of the flask. Mix by gently pipetting up and down 2 - 3 times.	Top up to double the original sample volume
	Place the flask (without cap) into the magnet and incubate.	RT for 5 minutes
8	Carefully pipette (do not pour) off the supernatant. Remove the flask, containing the isolated cells, from the magnet.	Discard supernatant
9	Repeat steps as indicated. Be sure to resuspend the cells from the side of the flask.	Steps 7 and 8 (total of 1 x 10-minute and 2 x 5-minute separations)
10	Resuspend cells in desired medium. Be sure to resuspend the cells from the side of the flask. Carefully pipette (do not pour) the cell suspension into a new tube or centrifuge bottle.	Use a new tube or centrifuge bottle*
11	Centrifuge sample; carefully aspirate and discard supernatant.	Centrifuge at 300 x g for 10 minutes at RT with low brake
	Resuspend to the desired cell concentration using recommended medium.	Isolated cells are ready for use

RT - room temperature (15 - 25°C)
* e.g. 50 mL (30 x 115 mm) conical tube (Catalog #38010) or 225 mL centrifuge bottle (Corning Catalog #352075)

Notes and Tips

ASSESSING PURITY

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

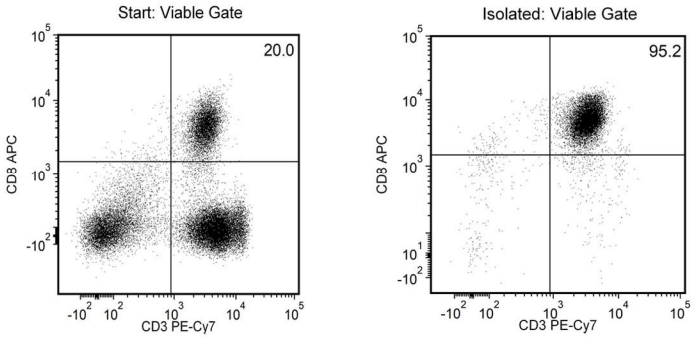
- Anti-Human CD8a Antibody, Clone RPA-T8 (Catalog #60022), or
- Anti-Human CD8a Antibody, Clone SK1 (Catalog #60125; partially blocked), or
- Anti-human CD8 antibody, clone HIT8a, or clone B9.11, or
- Anti-human CD8 antibody, clone LT8 (partially blocked)

One of the following methods can also be used:

- Use an alternative marker such as fluorochrome-conjugated Anti-Human CD3 Antibody, Clone UCHT1 (Catalog #60011).
- Use a fluorochrome-conjugated secondary antibody, such as Goat Anti-Mouse IgG (H+L) Antibody, Polyclonal (Catalog #60138).

- After addition of Cocktail and RapidSpheres™, mix the sample with a 25 mL or 50 mL serological pipette (e.g. Catalog #38005/38006).
  NOTE: Mixing can also be done by rotating or gently agitating the flask. Cap the flask first to prevent spillage.
- To remove the supernatant, gently sweep the pipette back and forth along the midline of the T-75 cm^2 flask while aspirating. Avoid touching the sides of the flask. Switch to a 10 mL or smaller serological pipette to collect the residual supernatant.

Data



Starting with washed or lysed leukapheresis samples, the CD8+ T cell content (CD3+CD8+) of the isolated fraction is typically 93.9 ± 4.9% (gated on viable cells, mean ± SD for the Easy 250 EasySep™ Magnet). In the above example, the purities of the start and final isolated fractions are 20.0% and 95.2%, respectively.