EasySep™ Human CD8 Positive Selection Kit II

Depletion protocol

For processing 1 x 10^9 cells

Catalog #17853

Document #10000031469 | Version 00

STEMCELL™ TECHNOLOGIES
Scientists Helping Scientists™ | WWW.STEMCELL.COM
TOLL FREE PHONE 1 800 667 0322 • PHONE +1 604 877 0713
INFO@STEMCELL.COM • TECHSUPPORT@STEMCELL.COM
FOR GLOBAL CONTACT DETAILS VISIT OUR WEBSITE

Description

Deplete CD8+ cells from fresh human peripheral blood mononuclear cells (PBMCs) or washed leukapheresis samples in as little as 18 minutes by immunomagnetic depletion.

- Fast and easy-to-use
- Up to 99% depletion
- No columns required

This kit targets CD8+ cells for depletion with antibodies recognizing the CD8 surface marker. Target 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 and are immediately available for downstream applications, such as flow cytometry, culture, or DNA/RNA extraction.

NOTE: Processing 1 x 10^9 cells using the depletion protocol requires two vials of EasySep™ Dextran RapidSpheres™ 50100. To purchase an additional vial, contact us at orders@stemcell.com.

Component Descriptions

COMPONENT NAME	COMPONENT #	QUANTITY	STORAGE	SHELF LIFE	FORMAT
EasySep™ Human CD8 Positive Selection Cocktail II	17853C	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 with 0.09% rHA. Includes an Fc receptor blocking antibody.
EasySep™ Dextran RapidSpheres™ 50100	50100	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.

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 samples, see www.stemcell.com/primarycells

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 red blood cell (RBC) lysis is necessary, lyse with Ammonium Chloride Solution (Catalog #07800). 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.

PERIPHERAL BLOOD

Prepare a PBMC suspension from whole blood by centrifugation over a density gradient medium (e.g. Lymphoprep™, Catalog #07811). For more rapid PBMC preparation, use the SepMate™ RUO (Catalog #86450/86415) or SepMate™ IVD* (Catalog #85450/85415) cell isolation tube.

After preparation, resuspend cells at 1 x 10^8 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).

Recommended Medium

EasySep™ Buffer (Catalog #20144) 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 Tables 1 and 2 for detailed instructions regarding the EasySep™ procedure for each magnet.

Table 1. EasySep™ Human CD8 Depletion 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.	1 x 10^8 cells/mL 0.25 - 2 mL	1 x 10^8 cells/mL 0.25 - 8 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 Cocktail to sample. NOTE: Do not vortex cocktail.	100 µL/mL of sample	100 µL/mL of sample
	Mix and incubate.	RT for 3 minutes	RT for 3 minutes
3	Vortex RapidSpheres™. NOTE: Particles should appear evenly dispersed.	30 seconds	30 seconds
4	Add RapidSpheres™ to sample.	50 µL/mL of sample	50 µL/mL of sample
	Mix and incubate.	RT for 3 minutes	RT for 3 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	• Top up to 5 mL for samples < 1 mL • Top up to 10 mL for samples ≥1 mL
	Place the tube (without lid) into the magnet and incubate.	RT for 3 minutes	RT for 3 minutes
6	Pick up the magnet, and in one continuous motion invert the magnet and tube,* pouring the depleted cell suspension into a new tube.	Use a new 5 mL tube	Use a new 14 mL tube
7	Add RapidSpheres™ to the depleted cell suspension.	50 µL/mL of original sample volume	50 µL/mL of original sample volume
	Mix and incubate.	RT for 3 minutes	RT for 3 minutes
8	Remove the tube from the magnet; place the new tube (without lid) into the magnet and incubate for a second separation.	RT for 3 minutes	RT for 3 minutes
9	Pick up the magnet, and in one continuous motion invert the magnet and tube,* pouring the depleted cell suspension into a new tube.	Use a new 5 mL tube	Use a new 14 mL tube
10	Remove the tube from the magnet; place the new tube (without lid) into the magnet and incubate for a third separation.	RT for 3 minutes	RT for 3 minutes
11	Pick up the magnet, and in one continuous motion invert the magnet and tube,* pouring the depleted 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 CD8 Depletion Protocol

		EASYSEP™ MAGNETS		
		EasyEights™ (Catalog #18103)		
STEP	INSTRUCTIONS	5 mL tube	14 mL tube	Easy 50 EasySep™ (Catalog #18002)
1	Prepare sample at the indicated cell concentration within the volume range.	1 x 10^8 cells/mL 0.1 - 2 mL	1 x 10^8 cells/mL 0.25 - 8 mL	1 x 10^8 cells/mL 5 - 35 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)	50 mL (30 x 115 mm) conical tube (e.g. Catalog #38010)
2	Add Cocktail to sample. NOTE: Do not vortex cocktail.	100 µL/mL of sample	100 µL/mL of sample	100 µL/mL of sample
	Mix and incubate.	RT for 3 minutes	RT for 3 minutes	RT for 3 minutes
3	Vortex RapidSpheres™. NOTE: Particles should appear evenly dispersed.	30 seconds	30 seconds	30 seconds
4	Add RapidSpheres™ to sample.	100 µL/mL of sample	100 µL/mL of sample	100 µL/mL of sample
	Mix and incubate.	RT for 3 minutes	RT for 3 minutes	RT for 3 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 2.5 mL	Top up to 5 mL for samples < 1 mL Top up to 10 mL for samples ≥ 1 mL	Top up to: • 10 mL for samples ≤ 5 mL • 20 mL for samples > 5 - 10 mL • 30 mL for samples > 10 - 15 mL • 40 mL for samples > 15 - 20 mL • 50 mL for samples > 20 - 35 mL
	Place the tube (without lid) into the magnet and incubate.	RT for 10 minutes	RT for 10 minutes	RT for 10 minutes
6	Carefully pipette** (do not pour) the depleted cell suspension into a new tube.	Use a new 5 mL tube	Use a new 14 mL tube	Use a new 50 mL tube
7	Add RapidSpheres™ to the depleted cell suspension.	100 µL/mL of original sample volume	100 µL/mL of original sample volume	100 µL/mL of original sample volume
	Mix and incubate.	RT for 3 minutes	RT for 3 minutes	RT for 3 minutes
8	Remove the tube from the magnet; place the new tube (without lid) into the magnet and incubate for a second separation.	RT for 10 minutes	RT for 10 minutes	RT for 10 minutes
9	Carefully pipette** (do not pour) the depleted cell suspension into a new tube.	Use a new 5 mL tube	Use a new 14 mL tube	Use a new 50 mL tube
10	Remove the tube from the magnet; place the new tube (without lid) into the magnet and incubate for a third separation.	RT for 5 minutes	RT for 5 minutes	RT for 5 minutes
11	Carefully pipette** (do not pour) the depleted cell suspension into a new tube.	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]).

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Notes and Tips

ASSESSING PURITY

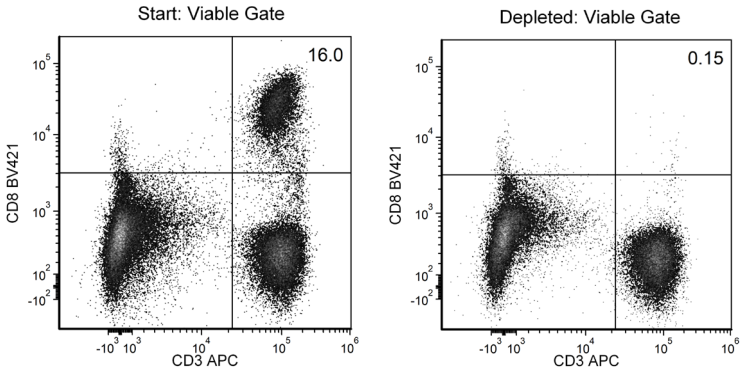
For purity assessment of CD8+ cells by flow cytometry, use one of the following fluorochrome-conjugated antibody clones:

- 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 alternative markers such as fluorochrome-conjugated Anti-Human CD3 Antibody, Clone UCHT1 (Catalog #60011) and Anti-Human CD4 Antibody, Clone OKT4 (Catalog #60016).
- Use a fluorochrome-conjugated secondary antibody, such as Goat Anti-Mouse IgG (H+L) Antibody, Polyclonal (Catalog #60138).

Data



Starting with a leukopheresis sample, the CD8+ cell content (based on viable cells) of the depleted fraction is typically 0.84 ± 0.87% (mean ± SD using “The Big Easy” EasySep™ Magnet). In the above example, the purities of the start and final depleted fractions are 16.0% and 0.15%, respectively.