EasySep™ Human CD45 Depletion Kit

Positive Selection

Catalog #18259

For processing 2 x 10^9 cells

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Description

Deplete CD45+ cells from fresh or previously frozen human peripheral blood mononuclear cells (PBMCs).

- Fast, easy-to-use and column-free
- Up to 4 log depletion of CD45+ cells
- Isolated cells are untouched

This kit targets CD45+ cells for removal with an antibody recognizing the CD45 cell surface marker. Unwanted 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. Isolated cells 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™ Human CD45 Depletion Cocktail	18259C.1	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.
EasySep™ Magnetic Nanoparticles Positive Selection	18150	2 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

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.

PERIPHERAL BLOOD

Prepare a PBMC suspension from whole blood by centrifugation over a density gradient medium (e.g. Lymphoprep™, Catalog #07801). For more rapid PBMC preparation, use the SepMate™ RUO (Catalog #86450/86415) or SepMate™ IVD* (Catalog #85450/85415) cell isolation tube.

If using previously frozen PBMCs, incubate the cells with DNase I Solution (Catalog #07900) at a concentration of 100 µg/mL at room temperature (15 - 25°C) for at least 15 minutes prior to labeling and separation. Filter aggregated suspensions through a 40 µm Cell Strainer (Catalog #27305) for optimal results.

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), RoboSep™ Buffer (Catalog #20104), or PBS containing 2% fetal bovine serum (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 for each magnet.

Table 1. EasySep™ Human CD45 Depletion Kit 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 0.1 - 2.5 mL NOTE: If starting with fewer than 1 x 10^7 cells, resuspend cells in 0.1 mL	1 x 10^8 cells/mL 0.25 - 8.5 mL NOTE: If starting with fewer than 2.5 x 10^7 cells, resuspend cells in 0.25 mL
	Add 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	Add Depletion Cocktail to sample.	50 µL/mL of cells	50 µL/mL of cells
	Mix and incubate.	RT for 15 minutes	RT for 15 minutes
3	Mix Magnetic Particles. NOTE: Particles should appear evenly dispersed.	Pipette up and down 5 times	Pipette up and down 5 times
4	Add Magnetic Particles to sample.	100 µL/mL of sample	100 µL/mL of sample
	Mix and incubate.	RT for 10 minutes	RT for 10 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 10 minutes	RT for 10 minutes
6	Pick up the magnet, and in one continuous motion invert the magnet and tube,* pouring the enriched cell suspension into a new tube.	Use new 5 mL tube	Use new 14 mL tube
7	Remove the tube from the magnet and place the new tube (without lid) into the magnet and incubate for a second separation.	RT for 10 minutes	RT for 10 minutes
8	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 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.

Directions for Use – Fully Automated RoboSep™ Protocol

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

Table 2. RoboSep™ Human CD45 Depletion Kit Protocol

STEP	INSTRUCTIONS	RoboSep™ (Catalog #20000 and #21000) 
1	Prepare sample at the indicated cell concentration within the volume range.	1 x 10^8 cells/mL 0.5 - 8.5 mL NOTE: If starting with fewer than 5 x 10^7 cells, resuspend cells in 0.5 mL
	Add sample to required tube.	14 mL (17 x 100 mm) polystyrene round-bottom tube (e.g. Corning Catalog #352057)
2	Select protocol.	• Human CD45 Depletion 18259-high purity • Human CD45 Depletion 18259-high recovery
3	Mix Magnetic Particles. NOTE: Particles should appear evenly dispersed.	Pipette up and down 5 times
4	Load the carousel.	Follow on-screen prompts
	Start the protocol.	Press the green “Run” button
5	Unload the carousel when the run is complete. Remove the tube containing the enriched cells.	Isolated cells are ready for use

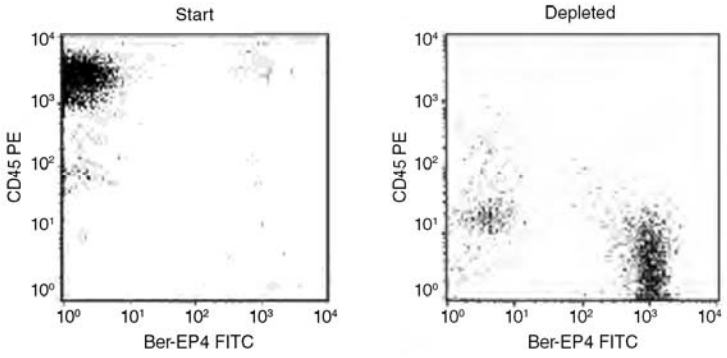
Notes and Tips

ASSESSING PURITY

For purity assessment of residual CD45+ cells by flow cytometry use one of the following fluorochrome-conjugated antibody clones:

- Anti-Human CD45 Antibody, Clone HI30 (Catalog #60018; partially blocked), or
- Anti-Human CD45 Antibody, Clone 2D1 (Catalog #60123; partially blocked)

Data



In the example above, CAMA cells were seeded into PBMCs at a starting frequency of 0.7% (99.3% CD45+). The CD45+ content of the depleted fraction is 1.5%, which is a 4.0 log depletion of CD45+ cells.

NOTE: Ber-EP4 is an antibody against an epithelial cell surface antigen expressed on CAMA cells.