EasySep™ Human Whole Blood CD45 Depletion Kit

Positive Selection

Catalog #18289

For processing 100 mL whole blood

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Description

Deplete CD45+ cells directly from HetaSep™-treated whole blood.

- 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 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 Whole Blood CD45 Depletion Cocktail	18289C	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	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.
HetaSep™	07806	1 x 20 mL	Store at 15 - 25°C.	Stable until expiry date (EXP) on label.	Hetastarch solution used for erythrocyte aggregation.

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.

WHOLE BLOOD USING HETASEP™ RED BLOOD CELL (RBC) SEDIMENTATION

1. Collect whole blood in a blood collection tube containing anticoagulant.
2. Use the minimum-sized tube for the total volume of HetaSep™ : blood sample. Add 1 part HetaSep™ to 5 parts blood and mix well.

- For smaller samples use: 14 mL (17 x 100 mm) polystyrene round-bottom tube (e.g. Corning Catalog #352057)
- For larger samples use: 50 mL polypropylene tubes (e.g. Corning Catalog #352070)

NOTE: For optimal results select tube sizes so that tubes can be at least 80% full, multiple tubes may be necessary. Nucleated cell recovery can decrease when using tubes less than half full due to the shorter sedimentation distance.

3. Centrifuge the sample at 50 x g for 5 minutes at room temperature (15 - 25°C) with the brake off. Remove sample from centrifuge and allow to sit undisturbed at room temperature for 5 minutes. This will allow further sedimentation of the RBCs and will improve nucleated cell recovery.

NOTE: Older samples will settle more slowly so centrifugation step at 200 x g may be required.

4. Harvest the supernatant containing the nucleated cells.
5. Top up the harvested fraction with recommended medium and centrifuge at 200 x g for 10 minutes at room temperature with the brake off.
6. Carefully remove the supernatant.
7. Resuspend cells in the recommended medium at 1/10th of the original starting volume (e.g. resuspend the cells recovered from 10 mL of whole blood in 1 mL of recommended medium).

WHOLE BLOOD USING RBC LYSIS

If RBC lysis is acceptable, lyse with Ammonium Chloride Solution (Catalog #07800).

1. Add 10 parts Ammonium Chloride Solution to 1 part whole blood.
2. Incubate for 15 minutes on ice.
3. Centrifuge at 200 x g for 10 minutes.
4. Discard supernatant, and resuspend the cells at 1/10th of the starting volume in recommended medium.

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.

Table 1. EasySep™ Human Whole Blood CD45 Depletion Kit Protocol

STEP	INSTRUCTIONS	 **"The Big Easy" (Catalog #18001)**
1	**Prepare HetaSep™-treated sample within the volume range.**	Up to 8.5 mL
	Add sample to required tube.	14 mL (17 x 100 mm) polystyrene round-bottom tube (e.g. Corning Catalog #352057)
2	**Add Depletion Cocktail to sample.**	100 µL/mL of sample
	Mix and incubate.	RT for 15 minutes
3	**Mix Magnetic Particles.** **NOTE: Particles should appear evenly dispersed.**	Pipette up and down more than 5 times
4	**Add Magnetic Particles to sample.**	100 µL/mL of sample
	Mix and incubate.	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 5 mL for samples < 2.5 mL • Top up to 10 mL for samples ≥ 2.5 mL
	Place the tube (without lid) into the magnet and incubate.	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.**	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 Whole Blood CD45 Depletion Kit Protocol

STEP	INSTRUCTIONS	RoboSep™ (Catalog #20000 and #21000) 
1	Prepare HetaSep™-treated sample within the volume range.	0.25 - 8.5 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 WB Depletion 18289-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.	Isolated cells are ready for use

Notes and Tips

OPTIMIZING RECOVERY

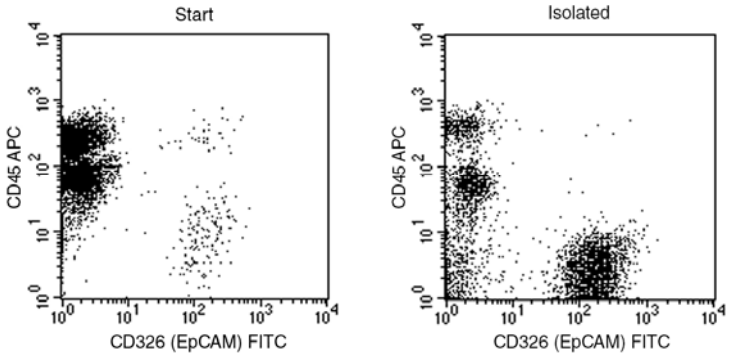
This no-lysis kit is designed for mid-range recovery of CD45- cells, along with 2 to 4 log depletion of CD45+ cells. If higher recovery of CD45- cells is desired, particles and cocktail may be titrated.

ASSESSING PURITY

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

- Anti-Human CD45 Antibody Clone HI30 (Catalog #60018) or Clone 2D1 (Catalog #60123), and
- Anti-Human CD235ab (Glycophorin A/B) Antibody, Clone HIR2 (Catalog #60111)

Data



Starting with human whole blood, the depletion of CD45+ cells typically ranges from 2 - 4 log. In the example above, CAMA cells were seeded into whole blood at a starting frequency of 1.7% (98.3% CD45+). The depletion of CD45+ cells was 2 log.

NOTE: Plots were gated on Glycophorin A negative cells to exclude residual RBCs. CD326 (EpCAM) is an antibody against an epithelial cell surface antigen expressed on CAMA cells.