EasySep™ RBC Depletion Reagent

For processing 100 mL whole blood

Catalog #18170

Negative Selection

Document #10000003345 | Version 03

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 red blood cells (RBCs) directly from human whole blood by immunomagnetic negative selection. This kit can also be used to deplete RBCs from other sample types (see Table 1).

The benefits of this kit include:
- 99.9% RBC depletion without the need for density gradient centrifugation, sedimentation, or lysis
- Fast, easy-to-use, and column-free
- Isolated cells are untouched

This kit targets RBCs for removal with antibodies recognizing specific cell surface markers. Unwanted cells are labeled with antibodies and magnetic particles, and separated without columns using an EasySep™ magnet. RBC-depleted nucleated cells are simply collected into a new tube and are immediately available for downstream applications such as flow cytometry, culture, or DNA/RNA extraction.

NOTE: This is the Product Information Sheet (PIS) for depleting RBCs from whole blood. If depleting RBCs from other sample types, refer to the applicable PIS Document Number (see Table 1).

Table 1. Applicable PIS Document Number for Other Sample Types

SAMPLE TYPE	PIS DOCUMENT NUMBER
Buffy coat	10000005628
Bone marrow	10000005629
Cord blood	10000005630
Leukapheresis	10000005627

Component Descriptions

Components may be shipped at room temperature (15 - 25°C) but should be stored as indicated below.

EasySep™ RBC Depletion Reagent (Catalog #18170)

COMPONENT NAME	COMPONENT #	QUANTITY	STORAGE	SHELF LIFE	FORMAT
EasySep™ RBC Depletion Reagent	18170	10 mL	Store at 2 - 8°C. Do not freeze.	Stable until expiry date (EXP) on label.	A suspension of magnetic particles and monoclonal antibodies in PBS.

PBS - phosphate-buffered saline

EasySep™ RBC Depletion Reagent for RoboSep™ (Catalog #18170RF)

COMPONENT NAME	COMPONENT #	QUANTITY	STORAGE	SHELF LIFE	FORMAT
EasySep™ RBC Depletion Reagent	18170C	4 x 2.5 mL	Store at 2 - 8°C.	Stable until expiry date (EXP) on label.	A suspension of magnetic particles and monoclonal antibodies in PBS.

PBS - phosphate-buffered saline

Sample Preparation

For best recovery, use unprocessed human whole blood. Recovery of the desired isolated cells decreases with samples that are older than 24 hours.

The volume of blood that can be processed depends on the EasySep™ magnet used for the isolation procedure. Blood samples must be placed in the required tube or flask to properly fit into the appropriate EasySep™ magnet.

To avoid loss of monocytes, EDTA must be added to the whole blood sample to a final concentration of 6 mM prior to labeling and separation (see step 2, Tables 2 - 5).

NOTE: An EDTA stock solution greater than 0.05 M is recommended to avoid overdiluting the start sample.

Recommended Medium

PBS containing 2% fetal bovine serum (FBS). 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 2 - 4 for detailed instructions regarding the EasySep™ procedure for each magnet.

Table 2. EasySep™ RBC Depletion Reagent Protocol for WHOLE BLOOD

		EASYSEP™ MAGNETS	
STEP	INSTRUCTIONS	 **EasySep™ (Catalog #18000)**	**"The Big Easy" (Catalog #18001)** 
1	**Prepare sample within the volume range.**	0.5 - 1 mL	1 - 5 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 EDTA to sample.**	At a final concentration of 6 mM EDTA	At a final concentration of 6 mM EDTA
3	**Dilute sample with recommended medium.**	Equal volume to sample	Equal volume to sample
4	**Vortex Depletion Reagent.** **NOTE: Reagent should appear evenly dispersed.**	30 seconds	30 seconds
5	**Add Depletion Reagent to sample and mix.**	50 µL/mL of original sample volume NOTE: No incubation, IMMEDIATELY proceed to next step	50 µL/mL of original sample volume NOTE: No incubation, IMMEDIATELY proceed to next step
6	**Place the tube (without lid) into the magnet and incubate.**	RT for 5 minutes	RT for 5 minutes
7	**Pick up the magnet, and in one continuous motion invert the magnet and tube, pouring the cell suspension* into a new tube.**	Use a new 5 mL tube	Use a new 14 mL tube
8	**Add Depletion Reagent to the new tube containing the depleted cells and mix.**	Use same volume as in step 5 NOTE: No incubation, IMMEDIATELY proceed to next step	Use same volume as in step 5 NOTE: No incubation, IMMEDIATELY proceed to next step
9	**Remove the tube from the magnet; Place the tube from step 8 (without lid) into the magnet and incubate for a second separation.**	RT for 5 minutes	RT for 5 minutes
10	**Pick up the magnet, and in one continuous motion invert the magnet and tube,† pouring the cell suspension into a new tube.**	Isolated cells are ready for use	Use a new 14 mL tube
11	**Remove the tube from the magnet; Place the tube from step 10 (without lid) into the magnet and incubate for a third separation.**	---	RT for 5 minutes
12	**Pick up the magnet, and in one continuous motion invert the magnet and tube,† pouring the cell suspension into a new tube.**	---	Isolated cells are ready for use

RT - room temperature (15 - 25°C)
* Following the first magnetic separation, the collected cells may contain a significant amount of RBCs and may look similar to the original unprocessed human whole blood sample.
† To minimize RBC contamination in the isolated cells, pour off the sample along a clean area of the tube (i.e. the opposite side to where the sample was poured in).

Table 3. EasySep™ RBC Depletion Reagent Protocol for WHOLE BLOOD

		EASYSEP™ MAGNETS			
STEP	INSTRUCTIONS	 EasyPlate™ (Catalog #18102)	 EasyEights™ (Catalog #18103) 5 mL tube	EasyEights™ (Catalog #18103) 14 mL tube 	Easy 50 (Catalog #18002) 
1	Prepare sample within the volume range.	0.1 mL	0.5 - 2 mL	1 - 6 mL	5 - 20 mL
	Add sample to required tube (or plate when using EasyPlate™ EasySep™ Magnet).	Round bottom, non-tissue culture-treated 96-well plate (e.g. Catalog #38018)	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 EDTA to sample.	At a final concentration of 6 mM EDTA	At a final concentration of 6 mM EDTA	At a final concentration of 6 mM EDTA	At a final concentration of 6 mM EDTA
3	Dilute sample with recommended medium.	Equal volume to sample	Equal volume to sample	Equal volume to sample	Equal volume to sample
4	Vortex Depletion Reagent. NOTE: Reagent should appear evenly dispersed.	30 seconds	30 seconds	30 seconds	30 seconds
5	Add Depletion Reagent to sample and mix.	50 µL/mL of original sample volume NOTE: No incubation, IMMEDIATELY proceed to next step	50 µL/mL of original sample volume NOTE: No incubation, IMMEDIATELY proceed to next step	50 µL/mL of original sample volume NOTE: No incubation, IMMEDIATELY proceed to next step	50 µL/mL of original sample volume NOTE: No incubation, IMMEDIATELY proceed to next step
6	Place the tube or plate (without lid) into the magnet and incubate.	RT for 3 minutes	RT for 5 minutes	RT for 5 minutes	RT for 10 minutes
7	Carefully pipette** (do not pour) the cell suspension into a new tube. NOTE: Collect the entire clear fraction from top to bottom. For optimal recovery, also collect a small volume of RBCs (up to 10% of the starting sample volume).	Use a new well of the 96-well plate	Use a new 5 mL tube	Use a new 14 mL tube	Use a new 50 mL tube
8	Add Depletion Reagent to the new tube containing the depleted cells and mix.	Use same volume as in step 5 NOTE: No incubation, IMMEDIATELY proceed to next step	Use same volume as in step 5 NOTE: No incubation, IMMEDIATELY proceed to next step	Use same volume as in step 5 NOTE: No incubation, IMMEDIATELY proceed to next step	Use same volume as in step 5 NOTE: No incubation, IMMEDIATELY proceed to next step
9	Remove the tube from the magnet; Place the tube from step 8 (without lid) into the magnet and incubate for a second separation.	RT for 3 minutes	RT for 5 minutes	RT for 5 minutes	RT for 10 minutes
10	Carefully pipette** (do not pour) the cell suspension into a new tube.	Use a new well of the 96-well plate	Use a new 5 mL tube	Use a new 14 mL tube	Use a new 50 mL tube
11	Remove the tube from the magnet; Place the tube from step 10 (without lid) into the magnet and incubate for a third separation.	RT for 3 minutes	RT for 5 minutes	RT for 5 minutes	RT for 10 minutes
12	Carefully pipette** (do not pour) the cell suspension into a new tube.	Isolated cells are ready for use	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]).

Table 4. EasySep™ RBC Depletion Reagent Protocol for WHOLE BLOOD

		EASYSEP™ MAGNETS
STEP	INSTRUCTIONS	**Easy 250 EasySep™ Magnet (Catalog #100-0821)** 
1	**Prepare sample within the volume range.**	20 - 125 mL
	Add sample to required flask.	T-75 cm^2 cell culture flask (i.e. Corning Catalog #353135)
2	**Add EDTA to sample.**	At a final concentration of 6 mM EDTA
3	**Dilute sample with recommended medium.**	Equal volume to sample
4	**Vortex Depletion Reagent.** **NOTE: Reagent should appear evenly dispersed.**	30 seconds
5	**Add Depletion Reagent to sample and mix with a 25 mL or 50 mL serological pipette.§** **NOTE: Mixing can also be done by rotating or gently agitating the flask. Cap the flask first to prevent spillage.**	50 µL/mL of original sample volume NOTE: No incubation, IMMEDIATELY proceed to next step
6	**Place the flask (without cap) into the magnet and incubate.**	RT for 10 minutes
7	**Carefully pipette*** (do not pour) the cell suspension into a new flask.** **NOTE: Collect the entire clear fraction from top to bottom. For optimal recovery, also collect a small volume of RBCs (up to 10% of the starting sample volume).**	Use a new T-75 cm^2 flask
8	**Add Depletion Reagent to the new flask containing the depleted cells and mix as described in step 5.**	Use same volume as in step 5 NOTE: No incubation, IMMEDIATELY proceed to next step
9	**Remove the flask from the magnet; Place the flask from step 8 (without cap) into the magnet and incubate for a second separation.**	RT for 10 minutes
10	**Carefully pipette*** (do not pour) the cell suspension into a new flask.**	Use a new T-75 cm^2 flask
11	**Remove the flask from the magnet; Place the flask from step 10 (without cap) into the magnet and incubate for a third separation.**	RT for 10 minutes
12	**Carefully pipette*** (do not pour) the cell suspension into a new tube or centrifuge bottle.‡**	Isolated cells are ready for use

RT - room temperature (15 - 25°C)

§ e.g. 25 mL (Catalog #38005) or 50 mL (Catalog #38006) serological pipette

*** To collect 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.

‡ e.g. 50 mL (30 x 115 mm) conical tube (Catalog #38010) or 225 mL centrifuge bottle (Corning Catalog #352075)

Directions for Use – Fully Automated RoboSep™ Protocol

See page 1 for Sample Preparation and Recommended Medium. Refer to Table 5 for detailed instructions regarding the RoboSep™ procedure.

NOTE: If using RoboSep™-S, ensure the software is at least v.1.2.0.2 and a carousel compatible with this product is installed. Contact us at techsupport@stemcell.com for more information.

Table 5. RoboSep™ RBC Depletion Reagent Protocol for WHOLE BLOOD

STEP	INSTRUCTIONS	RoboSep™ (Catalog #21000) 
1	**Prepare sample within the volume range.**	1 - 5 mL
	Add sample to required tube.	14 mL (17 x 95 mm) polystyrene round-bottom tube (e.g. Catalog #38008)
2	**Add EDTA to sample.**	At a final concentration of 6 mM EDTA
3	**Select protocol.**	Human RBC Depletion 18170 - WB CB BM
4	**Vortex Depletion Reagent.** **NOTE: Reagent should appear evenly dispersed.**	30 seconds
5	**Load the carousel.**	Follow on-screen prompts
	Start the protocol.	Press the green “Run” button
6	**Unload the carousel when the run is complete.**	Isolated cells are ready for use

Notes and Tips

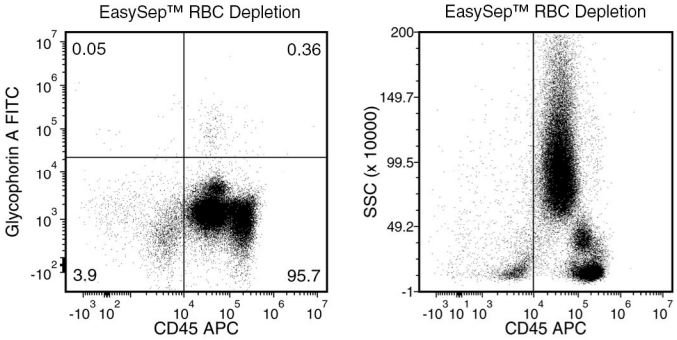
This product is not suitable for use with downstream magnetic positive selection products.

ASSESSING PURITY

For purity assessment of residual RBCs by flow cytometry, use the following fluorochrome-conjugated antibody clone:

- Anti-Human CD235ab (Glycophorin A/B), Clone HIR2 (Catalog #60111)

Data



Starting with human whole blood from normal healthy donors, the percentage of residual RBCs (Glycophorin A+/CD45-) following use of EasySep™ RBC Depletion Reagent is typically 2 ± 3 (mean ± SD; n = 31). In the above example, the residual RBC content is 0.05%.