EasySep™ RBC Depletion Reagent

Negative Selection

Catalog #18170 For processing 100 mL buffy coat

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Description

Deplete red blood cells (RBCs) directly from human buffy coat 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.

- This is the Product Information Sheet (PIS) for depleting RBCs from buffy coat. If depleting RBCs from other sample types, refer to the applicable PIS Document Number (see Table 1).

Table 1. Applicable Document Number for Other Sample Types

SAMPLE TYPE	DOCUMENT NUMBER
Whole blood	DX20431
Bone marrow	DX22481
Cord blood	DX22482
Leukapheresis	DX22483

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

BUFFY COAT

1. Add an equal volume of recommended medium to whole blood.
2. Centrifuge at 800 x g for 10 minutes at room temperature (15 - 25°C) with the brake off.
3. Remove the concentrated leukocyte band (this is the buffy coat), plus a small portion of the plasma and concentrated red blood cells (RBCs). The target is to concentrate the leukocytes approximately 5-fold while maintaining the same hematocrit (e.g. collect 2 mL of buffy coat when starting with 10 mL of whole blood).
4. Transfer buffy coat to the required tube.

To avoid loss of monocytes, EDTA must be added to the buffy coat sample to a final concentration of 6 mM prior to labeling and separation (see step 2, Tables 2 - 4).
NOTE: An EDTA stock solution greater than 0.05 M is recommended to avoid over diluting 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

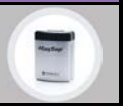
See page 1 for Sample Preparation and Recommended Medium. Refer to Tables 2 and 3 for detailed instructions regarding the EasySep™ procedure for each magnet.

Table 2. EasySep™ RBC Depletion Reagent Protocol for BUFFY COAT

		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 and 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.**	Use a new 5 mL tube	Use a new 14 mL tube
11	**Remove the tube from the magnet and place the tube from step 10 (without lid) into the magnet and incubate for a third separation.**	RT for 5 minutes	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	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 human buffy coat 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 BUFFY COAT

		EASYSEP™ MAGNETS		
		EasyEights™ (Catalog #18103)		Easy 50 (Catalog #18002) 
STEP	INSTRUCTIONS	5 mL tube 	14 mL tube 	
1	Collect sample within the volume range.	0.5 - 1 mL	1 - 5 mL	5 - 20 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 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
3	Dilute sample with recommended medium.	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
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
6	Place the tube (without lid) into the magnet and incubate.	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 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
9	Remove the tube from the magnet and 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	RT for 10 minutes
10	Carefully pipette*** (do not pour) the cell suspension into a new tube.	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 and place the tube from step 10 (without lid) into the magnet and incubate for a third separation.	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

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]).

Directions for Use – Fully Automated RoboSep™ Protocol

See page 1 for Sample Preparation and Recommended Medium. Refer to Table 4 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 4. RoboSep™ RBC Depletion Reagent Protocol for BUFFY COAT

STEP	INSTRUCTIONS	RoboSep™ (Catalog #21000) 
1	**Prepare sample within the volume range.**	2 - 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 - BC
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

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)