

EasySep™ HLA Chimerism Buffy Coat CD56 Positive Selection Kit



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For processing 30 mL buffy coat

Catalog #17875

Positive Selection

Document #1000003598 | Version 01

Description

Isolate highly purified CD56+ cells from buffy coat by immunomagnetic positive selection.

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

This kit targets CD56+ cells for positive selection with an antibody recognizing the CD56 surface marker. Desired cells are labeled with antibodies and magnetic particles, and separated without columns using an EasySep™ magnet. Unwanted cells are simply poured off, while desired cells remain in the tube. Isolated cells are immediately available for downstream applications such as flow cytometry, culture, or DNA/RNA extraction for lineage-specific chimerism analysis.

Component Descriptions

COMPONENT NAME	COMPONENT #	QUANTITY	STORAGE	SHELF LIFE	FORMAT
EasySep™ HLA Chimerism Buffy Coat CD56 Positive Selection Cocktail	17875C	2 x 0.75 mL	Store at 2 - 8°C. Do not freeze.	Stable until expiry date (EXP) on label.	A combination of monoclonal antibodies in PBS and 2% HPCD. Includes and Fc receptor blocking antibody.
EasySep™ Dextran RapidSpheres™ 50101	50101	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.
EasySep™ Red Blood Cell Lysis Buffer, 10X Concentrate	20110	1 x 10 mL	Store at 15 - 25°C.	Stable until expiry date (EXP) on label.	A 10X concentrated red blood cell lysis reagent.

HPCD - 2-hydroxypropyl-β-cyclodextrin; PBS - phosphate-buffered saline

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

Additional Reagent Stability Information

REAGENT NAME	STORAGE	SHELF LIFE
EasySep™ Red Blood Cell Lysis Buffer (1X dilution)	Store at 2 - 8°C. Do not freeze.	Stable for up to 3 months. Do not exceed the expiry date (EXP) of the original component.

Sample Preparation

For available fresh and frozen samples, see www.stemcell.com/primarycells.

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.
4. Transfer a maximum of 4.5 mL of buffy coat to the required tube (see Tables 1 - 3).

LEUKOREDUCTION SYSTEM CHAMBER

If processing leukoreduction system chamber (LRSC) samples, refer to the applicable PIS (Document #1000006419).

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 Tables 1 and 2 for detailed instructions regarding the EasySep™ procedure for each magnet.

Table 1. EasySep™ HLA Chimerism Buffy Coat CD56 Positive Selection Kit Protocol

		EASYSEP™ MAGNET
STEP	INSTRUCTIONS	<p>“The Big Easy” (Catalog #18001)</p> 
1	Prepare sample within the volume range.	0.5 - 4.5 mL
	Add sample to required tube.	14 mL (17 x 95 mm) polystyrene round-bottom tube (e.g. Catalog #38008)
2	Add 1X EasySep™ RBC Lysis Buffer to sample.	Equal volume to sample
	Mix and incubate.	RT for 5 minutes
3	Add Selection Cocktail to sample.	25 µL/mL of diluted sample
	Mix and incubate.	RT for 5 minutes
4	Vortex RapidSpheres™. NOTE: Particles should appear evenly dispersed.	30 seconds
5	Add RapidSpheres™ to sample.	25 µL/mL of diluted sample
	Mix and incubate.	RT for 5 minutes
6	Add recommended medium to top up the sample to the indicated volume. Mix by gently pipetting up and down 2 - 3 times.	<ul style="list-style-type: none"> • 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 5 minutes
7	Pick up the magnet, and in one continuous motion invert the magnet and tube,* pouring off the supernatant. Remove the tube from the magnet; this tube contains the isolated cells.	Discard supernatant
8	Repeat steps as indicated.	Steps 6 and 7, two more times (total of 3 x 5-minute separations)
9	Resuspend cells in desired medium. Be sure to collect cells from the sides of the 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.

Table 2. EasySep™ HLA Chimerism Buffy Coat CD56 Positive Selection Kit Protocol

		EASYSEP™ MAGNET	
		EasyEights™ (Catalog #18103)	
		14 mL tube	
			
1	Prepare sample within the volume range.	0.5 - 4.5 mL	
	Add sample to required tube.	14 mL (17 x 95 mm) polystyrene round-bottom tube (e.g. Catalog #38008)	
2	Add 1X EasySep™ RBC Lysis Buffer to sample.	Equal volume to sample	
	Mix and incubate.	RT for 5 minutes	
3	Add Selection Cocktail to sample.	25 µL/mL of diluted sample	
	Mix and incubate.	RT for 5 minutes	
4	Vortex RapidSpheres™. NOTE: Particles should appear evenly dispersed.	30 seconds	
5	Add RapidSpheres™ to sample.	25 µL/mL of diluted sample	
	Mix and incubate.	RT for 5 minutes	
6	Add recommended medium to top up the sample to the indicated volume. Mix by gently pipetting up and down 2 - 3 times.	<ul style="list-style-type: none"> • 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	
7	Carefully pipette** (do not pour) off the supernatant. Remove the tube from the magnet; this tube contains the isolated cells.	Discard supernatant	
8	Repeat steps as indicated.	Steps 6 and 7, two more times (total of 3 x 10-minute separations)	
9	Resuspend cells in desired medium. Be sure to collect cells from the sides of the tube.	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 the 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 3 for detailed instructions regarding the RoboSep™ procedure.

Table 3. RoboSep™ HLA Chimerism Buffy Coat CD56 Positive Selection Kit Protocol

STEP	INSTRUCTIONS	RoboSep™ (Catalog #20000 and #21000)
1	Prepare sample within the volume range.	0.5 - 4.5 mL
	Add sample to required tube.	14 mL (17 x 95 mm) polystyrene round-bottom tube (e.g. Catalog #38008)
2	Add 1X EasySep™ RBC Lysis Buffer to sample.	Equal volume to sample
	Mix and incubate.	RT for 5 minutes
3	Select protocol. NOTE: Enter volume.	HLA Chimerism CD56 BC Positive Selection 17875 NOTE: Enter diluted sample volume.
4	Vortex RapidSpheres™. NOTE: Particles 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. Remove the tube containing the isolated cells and resuspend in desired medium. Be sure to collect cells from the sides of the tube.	Isolated cells are ready for use

Notes and Tips

EASYSEPTM RED BLOOD CELL LYSIS BUFFER

EasySep™ Red Blood Cell Lysis Buffer is supplied as a 10X concentrate. Prepare 1X lysis buffer at least 1 hour before use by adding 1 part 10X lysis buffer to 9 parts distilled or Type 1 water. Mix gently and completely before use.

*Type I water refers to ultrapure water suitable for use in analytical procedures. It is defined by the American Society for Testing and Materials (ASTM) as having a resistivity of > 18 MΩ-cm, a conductivity of < 0.056 µS/cm, and < 50 ppb of total organic carbons (TOC).

ASSESSING PURITY

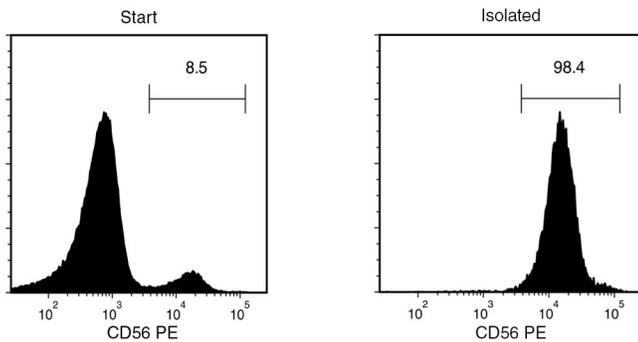
For purity assessment of CD56+ cells by flow cytometry use one of the following fluorochrome-conjugated antibody clones:

- Anti-Human CD56 Antibody (NCAM), Clone HCD56 (Catalog #60021), CMSSB, or NCAM16.2 (all partially blocked)

The following method can also be used:

- Use a fluorochrome-conjugated secondary antibody, such as Goat Anti-Mouse IgG (H+L) Antibody, Polyclonal (Catalog #60138).

Data



Starting with freshly prepared buffy coat, the CD56+ cell content of the isolated fraction typically ranges from 95.8 - 99.5% (gated on CD45+ using "The Big Easy" EasySep™ Magnet). In the above example, the purities of the start and final isolated fractions are 8.5% and 98.4%, respectively.

NOTE: RBCs were removed from the start sample by lysis prior to flow cytometry.

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