EasySep™

Positive Selection

Catalog #18085

EasySep™ Human Buffy Coat CD56 Positive Selection Kit

For processing 30 mL buffy coat

STEMCELL™ TECHNOLOGIES

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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

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Description

Isolate highly purified CD56+ cells from buffy coat samples 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.

Component Descriptions

COMPONENT NAME	COMPONENT #	QUANTITY	STORAGE	SHELF LIFE	FORMAT
EasySep™ Human Buffy Coat CD56 Positive Selection Cocktail	18085C.1	3 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. Includes an Fc receptor blocking antibody.
EasySep™ Magnetic Nanoparticles Positive Selection	18150	3 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.

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.

   NOTE: The sample must be washed before use to remove donor-specific soluble serum factor(s) that can cause cross-linking with magnetic nanoparticles.
2. Centrifuge at 200 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 Table 1).

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 Buffy Coat CD56 Positive Selection Kit Protocol

		"THE BIG EASY" EASYSEP™ MAGNET (CATALOG #18001)
STEP	INSTRUCTIONS	**Buffy Coat** 
1	**Prepare sample within the volume range.**	Up to 4.5 mL
	Add sample to required tube.	14 mL (17 x 100 mm) polystyrene round-bottom tube (e.g. Corning Catalog #352057)
2	**Add 1X EasySep™ RBC Lysis Buffer to sample.**	Equal volume to sample
3	**Add Selection Cocktail to sample.**	50 µL/mL of diluted sample
	Mix and incubate.	RT for 15 minutes
4	**Mix Magnetic Particles.** **NOTE: Particles should appear evenly dispersed.**	Pipette up and down more than 5 times
5	**Add Magnetic Particles to sample.**	50 µL/mL of diluted sample
	Mix and incubate.	RT for 10 minutes
6	**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
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	**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 5 minutes
9	**Repeat steps as indicated.**	Steps 7 and 8 (total of 1 x 10-minute and 2 x 5-minute separations)
10	**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.

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 Buffy Coat CD56 Positive Selection Kit Protocol

STEP	INSTRUCTIONS	RoboSep™ (Catalog #20000 and #21000) 
1	**Prepare sample within the volume range.**	0.25 - 4.5 mL
	Add sample to required tube.	14 mL (17 x 100 mm) polystyrene round-bottom tube (e.g. Corning Catalog #352057)
2	**Add 1X EasySep™ RBC Lysis Buffer to sample.**	Equal volume to sample
3	**Select protocol.**	Human CD56 Buffy Coat Positive Selection 18085-high purity
4	**Mix Magnetic Particles.** **NOTE: Particles should appear evenly dispersed.**	Pipette up and down more than 5 times
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

EASYSEP™ RED BLOOD CELL LYSIS BUFFER

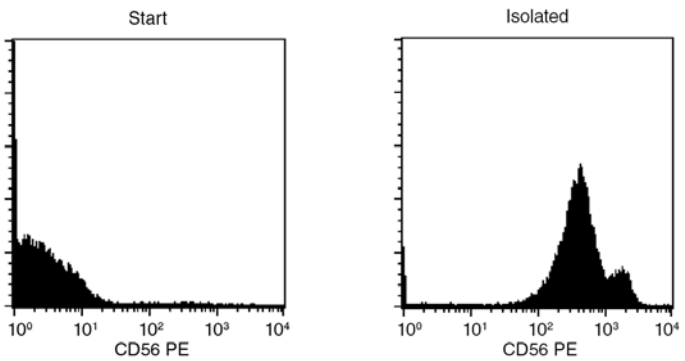
EasySep™ Red Blood Cell Lysis Buffer is supplied as a 10X concentrate. Prepare 1X lysis buffer at least 1 hour before use by adding 1 part 10X lysis buffer to 9 parts distilled or Type 1 water. Mix gently and completely before use.

ASSESSING PURITY

For purity assessment of CD56+ cells by flow cytometry use one of the following fluorochrome-conjugated antibody clones:
- Anti-human CD56 antibody, clone NCAM16.2, or
- Anti-Human CD56 (NCAM) Antibody, Clone HCD56 (Catalog #60021)

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 buffy coat, the CD56+ cell content of the isolated fraction typically ranges from 89.7 - 99.8%. In the above example, the purities of the start and final isolated fractions are 3.9% and 99.4%, respectively.