EasySep™ Human CD56 Positive Selection Kit II

For processing 1 x 10^9 cells

Catalog #17855
#17855RF RoboSep™

Positive Selection

Document #10000005559 | Version 04

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

Isolate highly purified CD56+ cells from fresh or previously frozen human peripheral blood mononuclear cells (PBMCs) in as little as 18 minutes by immunomagnetic positive selection.

- Fast and easy-to-use
- Up to 98% purity
- No columns required

This kit targets CD56+ cells for positive selection with antibodies 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.

- This is the Product Information Sheet (PIS) for isolating CD56+ cells from human PBMCs. If isolating CD56+ cells from human muscle cultures, refer to the applicable PIS (Document #10000000694).

Component Descriptions

COMPONENT NAME	COMPONENT #	QUANTITY	STORAGE	SHELF LIFE	FORMAT
EasySep™ Human CD56 Positive Selection Cocktail II	17855C	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 with 0.09% rHA. Includes an Fc receptor blocking antibody.
EasySep™ Dextran RapidSpheres™ 50100	50100	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.

PBS - phosphate-buffered saline; rHA - recombinant human albumin

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.

PERIPHERAL BLOOD

Prepare a mononuclear cell (MNC) suspension from whole peripheral blood by centrifugation over a density gradient medium (e.g. Lymphoprep™; Catalog #07811). For more rapid MNC preparation, use the SepMate™ RUO (Catalog #86450/86415) or SepMate™ IVD* (Catalog #85450/85415) cell isolation tube.

If using previously frozen MNCs, incubate the cells with DNase I Solution (Catalog #07900) at a concentration of 100 µg/mL at room temperature (15 - 25°C) for at least 15 minutes prior to labeling and separation. Filter aggregated suspensions through a 37 µm cell strainer (Catalog #27215) for optimal results.

After preparation, resuspend cells at 1 x 10^8 cells/mL in recommended medium.

*SepMate™ IVD is only available in select regions where it is registered as an In Vitro Diagnostic (IVD) device for the isolation of MNCs from whole blood or bone marrow by density gradient centrifugation. In all other regions, SepMate™ is available for research use only (RUO).

LYSED LEUKAPHERESIS

1. Add 4 parts Ammonium Chloride Solution (Catalog #07800) to 1 part leukapheresis sample.

   NOTE: If working with large volumes (> 20 mL), concentrate the leukapheresis sample first by centrifuging at 300 x g for 10 minutes. Remove the supernatant and resuspend the cells in 1/10th of the original leukapheresis volume with recommended medium (e.g. for 30 mL of cells, resuspend in 3 mL of recommended medium and add 12 mL of Ammonium Chloride Solution). For small volumes (≤ 20 mL), add Ammonium Chloride Solution directly to the leukapheresis sample.
2. Incubate on ice for 15 minutes.
3. Wash the cells by topping up the tube with recommended medium. Centrifuge at 300 x g for 10 minutes at room temperature (15 - 25°C). Remove the supernatant.

4. OPTIONAL (FOR PLATELET REMOVAL):

   a. Wash the cells by topping up the tube with recommended medium. Centrifuge the cells at 120 x g for 10 minutes at room temperature with the brake off. Carefully remove the supernatant.

   b. Repeat step 4a one or more times until most of the platelets have been removed (indicated by a clear supernatant).

5. Resuspend the cells at 1 x 10^8 cells/mL in recommended medium.

   NOTE: Working with lysed leukapheresis samples is recommended for optimal performance. Alternatively, washed leukapheresis samples may be used (see below) for faster sample processing, but a reduction in performance may be observed.

WASHED LEUKAPHERESIS

Wash the peripheral blood leukapheresis sample by adding an equivalent volume of recommended medium or PBS containing 2% fetal bovine serum (FBS). Centrifuge at 300 x g for 10 minutes at room temperature (15 - 25°C). If platelet removal is necessary, centrifuge at 120 x g for 10 minutes with the brake off. Remove the supernatant and resuspend the cells at 1 x 10^8 cells/mL in recommended medium.

Recommended Medium

EasySep™ Buffer (Catalog #20144), RoboSep™ Buffer (Catalog #20104), or PBS containing 2% FBS and 1 mM EDTA. Medium should be free of Ca++ and Mg++.

Directions for Use – Manual EasySep™ Protocols

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

Table 1. EasySep™ Human CD56 Positive Selection Kit II Protocol

		EASYSEP™ MAGNETS	
STEP	INSTRUCTIONS	 **EasySep™ (Catalog #18000)**	**"The Big Easy" (Catalog #18001)** 
1	**Prepare sample at the indicated cell concentration within the volume range.**	1 x 10^8 cells/mL 0.1 - 1 mL	1 x 10^8 cells/mL 0.5 - 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 Selection Cocktail to sample.** **NOTE: Do not vortex cocktail.**	100 µL/mL of sample	100 µL/mL of sample
	Mix and incubate.	RT for 3 minutes	RT for 3 minutes
3	**Vortex RapidSpheres™.** **NOTE: Particles should appear evenly dispersed.**	30 seconds	30 seconds
4	**Add RapidSpheres™ to sample.**	100 µL/mL of sample	100 µL/mL of sample
	Mix and incubate.	RT for 3 minutes	RT for 3 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 2.5 mL	• Top up to 5 mL for samples ≤ 1 mL • Top up to 10 mL for samples > 1 mL
	Place the tube (without lid) into the magnet and incubate.	RT for 3 minutes	RT for 3 minutes
6	**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	Discard supernatant
7	**Repeat steps as indicated.§**	Steps 5 and 6, three more times (total of 4 x 3-minute separations)	Steps 5 and 6, three more times (total of 4 x 3-minute separations)
8	**Resuspend cells in desired medium. Be sure to collect cells from the sides of the tube.**	Isolated cells are ready for use	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.

§ For increased recovery, see optional Table 2.

Table 2. Optional: EasySep™ Human CD56 Positive Selection Kit II Protocol for Increased Recovery

NOTE: Using this protocol will increase recovery, but it may decrease purity.

		EASYSEP™ MAGNETS	
STEP	INSTRUCTIONS	 **EasySep™ (Catalog #18000)**	**"The Big Easy" (Catalog #18001)** 
1	Prepare sample at the indicated cell concentration within the volume range.	1 x 10^8 cells/mL 0.1 - 1 mL	1 x 10^8 cells/mL 0.5 - 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 Selection Cocktail to sample. NOTE: Do not vortex cocktail.	100 µL/mL of sample	100 µL/mL of sample
	Mix and incubate.	RT for 3 minutes	RT for 3 minutes
3	Vortex RapidSpheres™. NOTE: Particles should appear evenly dispersed.	30 seconds	30 seconds
4	Add RapidSpheres™ to sample.	100 µL/mL of sample	100 µL/mL of sample
	Mix and incubate.	RT for 3 minutes	RT for 3 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 2.5 mL	• Top up to 2.5 mL for samples ≤ 1 mL • Top up to 5 mL for samples > 1 - 2 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	RT for 5 minutes
6	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	Discard supernatant
7	Repeat steps as indicated.	Steps 5 and 6, three more times (total of 4 x 5-minute separations)	Steps 5 and 6, three more times (total of 4 x 5-minute separations)
8	Resuspend cells in desired medium. Be sure to collect cells from the sides of the tube.	Isolated cells are ready for use	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 3. EasySep™ Human CD56 Positive Selection Kit II Protocol

		EASYSEP™ MAGNETS		
STEP	INSTRUCTIONS	 EasyPlate™ (Catalog #18102)	 EasyEights™ (Catalog #18103) 5 mL tube	EasyEights™ (Catalog #18103) 14 mL tube 
1	Prepare sample at the indicated cell concentration within the volume range.	1 x 10^8 cells/mL 0.05 - 0.2 mL	1 x 10^8 cells/mL 0.25 - 1 mL	1 x 10^8 cells/mL 0.5 - 5 mL
	Add sample to required tube.	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)
2	Add Selection Cocktail to sample. NOTE: Do not vortex cocktail.	100 µL/mL of sample	100 µL/mL of sample	100 µL/mL of sample
	Mix and incubate.	RT for 3 minutes	RT for 3 minutes	RT for 3 minutes
3	Vortex RapidSpheres™. NOTE: Particles should appear evenly dispersed.	30 seconds	30 seconds	30 seconds
4	Add RapidSpheres™ to sample.	100 µL/mL of sample	100 µL/mL of sample	100 µL/mL of sample
	Mix and incubate.	RT for 3 minutes	RT for 3 minutes	RT for 3 minutes
5	Add recommended medium to top up sample to the indicated volume. Mix by gently pipetting up and down 2 - 3 times.	Top up to 0.25 mL	Top up to 2.5 mL	• Top up to 5 mL for samples ≤ 1 mL • Top up to 10 mL for samples > 1 mL
	Place the tube or plate (without lid) into the magnet and incubate.	RT for 5 minutes	RT for 10 minutes	RT for 10 minutes
6	Carefully pipette** (do not pour) off the supernatant. Remove the tube or plate, containing the isolated cells, from the magnet.	Discard supernatant	Discard supernatant	Discard supernatant
7	Repeat steps as indicated.	Steps 5 and 6, two more times (total of 3 x 5-minute separations)	Steps 5 and 6, two more times (total of 3 x 10-minute separations)	Steps 5 and 6, two more times (total of 3 x 10-minute separations)
8	Resuspend cells in desired medium. Be sure to collect cells from the sides of the 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 and 2 for Sample Preparation and Recommended Medium. Refer to Table 4 for detailed instructions regarding the RoboSep™ procedure.

Table 4. RoboSep™ Human CD56 Positive Selection Kit II Protocol

STEP	INSTRUCTIONS	RoboSep™ (Catalog #21000) 
1	Prepare sample at the indicated cell concentration within the volume range.	1 x 10^8 cells/mL 0.5 - 8 mL
	Add sample to required tube.	14 mL (17 x 95 mm) polystyrene round-bottom tube (e.g. Catalog #38008)
2	Select protocol.	Human CD56 Positive Selection II 17855
3	Vortex RapidSpheres™. NOTE: Particles should appear evenly dispersed.	30 seconds
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. 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

ASSESSING PURITY

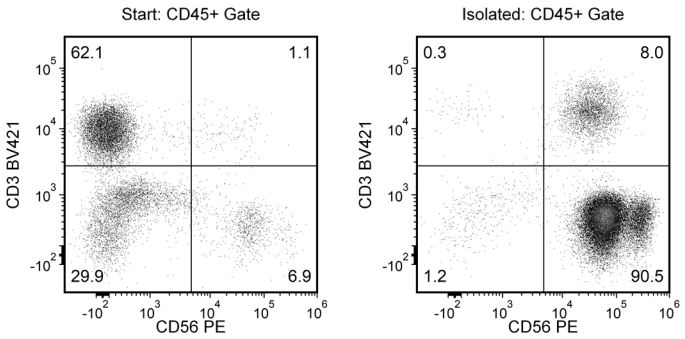
For purity assessment of CD56+ cells by flow cytometry, use one of the following fluorochrome-conjugated antibody clones:

- Anti-Human CD56 Antibody, Clone HDC56 (Catalog #60021; partial blocking), or
- Anti-human CD56 antibody, clone CMSSB (partial blocking), or
- Anti-human CD56 antibody, clone NCAM16.2 (partial blocking)

The following method can also be used:

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

Data



Starting with human PBMCs, the CD56+ cell content of the isolated fraction is typically 96.3 ± 2.4% (mean ± SD using the purple EasySep™ Magnet). In the above example, the purities of the start and final isolated fractions are 8.0% and 98.5%, respectively.