EasySep™

Positive Selection

Catalog #18651

EasySep™ Human CD15 Positive Selection Kit

For processing 1 x 10^9 cells

STEMCELL™ TECHNOLOGIES

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Description

Isolate highly purified myeloid cells (CD15+) from whole blood by immunomagnetic positive selection.

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

This kit targets myeloid cells for positive selection with an antibody recognizing the CD15 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 CD15 Positive Selection Cocktail	18651C	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.
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

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

Collect whole blood in a blood collection tube containing anticoagulant. A nucleated cell suspension can be prepared by lysing red blood cells (RBCs) using Ammonium Chloride Solution (Catalog #07800). Refer to the product information sheet for Ammonium Chloride Solution (Document #296212) for a lysis protocol.

Alternatively, RBCs can be lysed after density gradient separation.

1. Collect whole blood in a blood collection tube containing anticoagulant.
2. Carefully perform a standard density gradient separation (e.g. using Lymphoprep™; Catalog #07801). Do not use SepMate™.
3. Remove and discard the plasma layer, the band of mononuclear cells and the density gradient medium leaving the RBC pellet intact.
4. Add Ammonium Chloride Solution (Catalog #07800) to the RBC pellet and mix well.
5. Incubate on ice for 10 minutes then centrifuge at 500 x g for 10 minutes.
6. Discard supernatant and wash pellet with cold recommended medium, centrifuging at 120 x g for 10 minutes.
7. Discard supernatant and resuspend 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% 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™ Human CD15 Positive Selection Kit 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 0.25 - 1 mL	1 x 10^8 cells/mL 0.5 - 8 mL
2	**Add sample to required tube.**	5 mL (12 x 75 mm) polystyrene round-bottom tube (e.g. Corning Catalog #352058)	14 mL (17 x 100 mm) polystyrene round-bottom tube (e.g. Corning Catalog #352057)
3	**Add Selection Cocktail to sample.**	100 µL/mL of sample	100 µL/mL of sample
	Mix and incubate.	RT for 3 minutes	RT for 3 minutes
4	**Vortex RapidSpheres™.**	30 seconds	30 seconds
5	**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
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 2.5 mL	• Top up to 5 mL for samples < 2 mL • Top up to 10 mL for samples ≥ 2 mL
	Place the tube (without lid) into the magnet and incubate.	RT for 3 minutes	RT for 3 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	Discard supernatant
8	**Repeat steps as indicated.**	Steps 6 and 7 (total of 2 x 3-minute separations)	Steps 6 and 7** (total of 2 x 3-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	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 start samples > 5 mL, purity may be improved by one additional 3-minute separation. NOTE: This will improve purity but may reduce recovery.

Table 2. EasySep™ Human CD15 Positive Selection Kit Protocol

		EASYSEP™ MAGNETS	
		EasyEights™ (Catalog #18103)	
STEP	INSTRUCTIONS	5 mL tube	14 mL tube
1	Prepare sample at the indicated cell concentration within the volume range.	1 x 10^8 cells/mL 0.25 - 1 mL	1 x 10^8 cells/mL 0.5 - 8 mL
2	Add sample to required tube.	5 mL (12 x 75 mm) polystyrene round-bottom tube (e.g. Corning Catalog #352058)	14 mL (17 x 100 mm) polystyrene round-bottom tube (e.g. Corning Catalog #352057)
3	Add Selection Cocktail to sample.	100 µL/mL of sample	100 µL/mL of sample
	Mix and incubate.	RT for 3 minutes	RT for 3 minutes
4	Vortex RapidSpheres™.	30 seconds	30 seconds
5	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
6	Add recommended medium to top up 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 < 2 mL • Top up to 10 mL for samples ≥ 2 mL
	Place the tube (without lid) into the magnet and incubate.	RT for 10 minutes	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	Discard supernatant
8	Repeat steps as indicated.	Steps 6 and 7 (total of 2 x 10-minute separations)	Steps 6 and 7 (total of 2 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	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™ 5 mL tube use a 2 mL serological pipette and for the EasyEights™ 14 mL tube use a 10 mL serological pipette).

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™ EasySep™ Human CD15 Positive Selection Kit Protocol

STEP	INSTRUCTIONS	RoboSep™ (Catalog #20000 and #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 100 mm) polystyrene round-bottom tube (e.g. Corning Catalog #352057)
2	Select protocol.	Human CD15 Positive Selection 18651
3	Vortex RapidSpheres™.	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

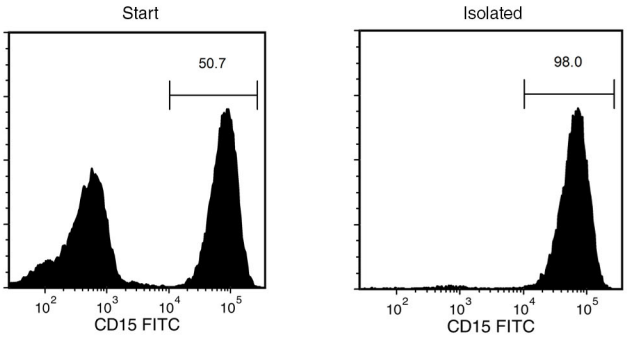
Notes and Tips

ASSESSING PURITY

For purity assessment by flow cytometry use the following fluorochrome-conjugated antibody clones:

- Anti-human CD15 antibody, clone HI98, and
- Anti-Human CD45 Antibody, Clone HI30 (Catalog #60018)

Data



Starting with lysed whole blood, the CD15+ cell content of the isolated fraction is typically 98.8 ± 0.8% (gated on CD45+ cells; mean ± SD). In the above example, the purities of the start and final isolated fractions are 50.7% and 98.0%, respectively.