EasySep™ Human CD33 Positive Selection Kit

Positive Selection

Catalog #18257   For processing 1 x 10^9 cells

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Description

Isolate highly purified CD33+ cells from whole blood or bone marrow by immunomagnetic positive selection.

· Fast and easy-to-use
· Up to 97% purity
· No columns required

This kit targets CD33+ cells for positive selection with an antibody recognizing the CD33 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 CD33 Positive Selection Cocktail	18257C.1	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™ Whole Blood Magnetic Nanoparticles	18180	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 or BONE MARROW

A nucleated cell suspension can be prepared with red blood cells by lysis using Ammonium Chloride Solution (Catalog #07800).

Alternatively, cells can be prepared by centrifugation over RosetteSep™ DM-M Density Medium (Catalog #15725). For more rapid PBMC preparation, use the SepMate™ RUO (Catalog #86450/86415) or SepMate™ IVD* (Catalog #85450/85415) cell isolation tube.

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 mononuclear cells (MNCs) from whole blood or bone marrow by density gradient centrifugation. In all other regions SepMate™ is available for research use only (RUO).

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 for each magnet.

Table 1. EasySep™ Human CD33 Positive Selection Kit Protocol

STEP	INSTRUCTIONS	EASYSEP™ MAGNETS	
		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 - 2.5 mL NOTE: If starting with fewer than 1 x 10^7 cells, resuspend cells in 0.1 mL	1 x 10^8 cells/mL 0.25 - 8 mL NOTE: If starting with fewer than 2.5 x 10^7 cells, resuspend cells in 0.25 mL
	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)
2	**Add Selection Cocktail to sample.**	100 µL/mL of sample	100 µL/mL of sample
	Mix and incubate.	RT for 15 minutes	RT for 15 minutes
3	**Mix Magnetic Particles.** NOTE: Particles should appear evenly dispersed.	Pipette up and down more than 5 times	Pipette up and down more than 5 times
4	**Add Magnetic Particles to sample.**	100 µL/mL of sample	100 µL/mL of sample
	Mix and incubate.	RT for 10 minutes	RT for 10 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 10 minutes	RT for 10 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	**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 15 minutes	RT for 15 minutes
8	**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
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.

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 CD33 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 0.25 - 8 mL NOTE: If starting with fewer than 2.5 x 10^7 cells, resuspend cells in 0.25 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 CD33 Positive Selection 18257–high purity
3	**Mix Magnetic Particles.** **NOTE: Particles should appear evenly dispersed.**	Pipette up and down more than 5 times
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

The EasySep™ Human CD33 Positive Selection Cocktail uses an anti-CD33 antibody clone that may block some anti-CD33 antibody clones used to assess purity by flow cytometry. For purity assessment of CD33+ cells by flow cytometry use the following fluorochrome-conjugated antibody clones:

· Anti-Human CD66b Antibody, Clone G10F5 (Catalog #60086), and
· Anti-Human CD14 Antibody, Clone M5E2 (Catalog #60004) or Clone MoP9 (Catalog #60124)

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
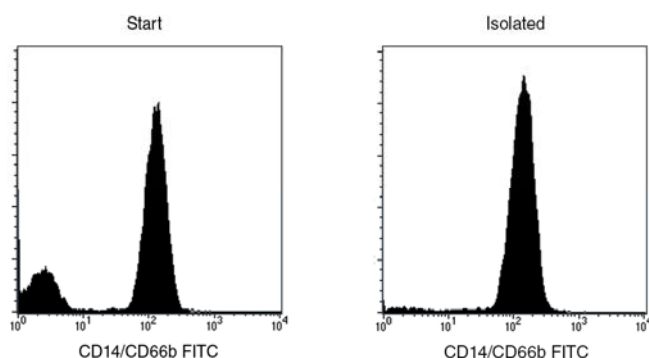
Starting with ammonium chloride-lysed peripheral blood, the CD33+ cell content of the isolated fraction typically ranges from 93 - 97% (as assessed by labeling with CD14 and CD66b). In the above example, the purities of the start and final isolated fractions are 76.2% and 96.7%, respectively.