+EasySep™

EasySep™ Human CD19 Positive Selection Kit

Positive Selection

Catalog #18054   For processing 1 x 10^9 cells

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Description

Isolate highly purified CD19+ cells from fresh or previously frozen human peripheral blood mononuclear cells (PBMCs) by immunomagnetic positive selection.

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

This kit targets CD19+ cells for positive selection with an antibody recognizing the CD19 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™ CD19 Positive Selection Cocktail	18054C.2	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. Includes an Fc receptor blocking antibody.
EasySep™ Magnetic Nanoparticles Positive Selection	18150	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

Prepare a PBMC suspension from whole peripheral blood by centrifugation over a density gradient medium (e.g. Lymphoprep™, Catalog #07801). For more rapid PBMC preparation, use the SepMate™ RUO (Catalog #86450/86415) or SepMate™ IVD* (Catalog #85450/85415) cell isolation tube.

If using previously frozen PBMCs, 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 40 μm Cell Strainer (Catalog #27305) 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 mononuclear cells (MNCs) from whole blood or bone marrow by density gradient centrifugation.

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 CD19 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 0.1 - 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.**	Pipette up and down more than 5 times	Pipette up and down more than 5 times
4	**Add Magnetic Particles to sample.****	50 µL/mL of sample	50 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 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, two more times (total of 3 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.
** To improve recovery, the Magnetic Particle addition can be increased to 100 µL/mL and the magnetic separation can be reduced by 1 separation to either 3 x 5-minute or 2 x 5-minute separations, depending on the magnet used.

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 CD19 Positive Selection 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 - 8.5 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 CD19 Positive Selection 18054-high purity • Human CD19 Positive Selection 18054-high recovery
3	**Mix Magnetic Particles.**	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

For purity assessment of CD19+ cells by flow cytometry use one of the following fluorochrome-conjugated antibody clones:
· Anti-Human CD19 Antibody, Clone HIB19 (Catalog #60005; partially blocked), or
· Anti-human CD19 antibody, clone 4G7 or FMV63 (partially blocked)

One of the following methods can also be used:
· Use an alternative marker such as fluorochrome-conjugated Anti-Human CD20 Antibody, Clone 2H7 (Catalog #60008). This may underestimate the CD19+ purity by up to 15%.
· Use a fluorochrome-conjugated secondary antibody, such as Goat Anti-Mouse IgG (H+L) Antibody, Polyclonal (Catalog #60138).

Data
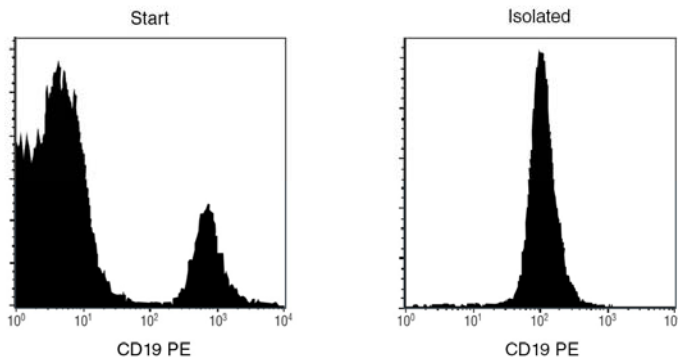
Starting with fresh PBMCs, the CD19+ cell content of the isolated fraction typically ranges from 97 - 99%. In the above example, the purities of the start and final isolated fractions are 8.3% and 98.5%, respectively.