EasySep™

Positive Selection

Catalog #18184HLA

EasySep™ HLA Whole Blood B Cell Positive Selection Kit

For processing 60 mL whole blood

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Description

Isolate highly purified B cells from fresh human whole blood by immunomagnetic positive selection.

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

This kit targets B cells for positive selection with antibodies recognizing the CD19 and CD20 surface markers. 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™ HLA WB B Cell Positive Selection Cocktail	18184HC	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™ Whole Blood Magnetic Particles	18180H	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 original component.

Sample Preparation

For available fresh and frozen samples, see www.stemcell.com/primarycells.

WHOLE BLOOD

Collect whole blood in a blood collection tube containing anticoagulant.

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™ HLA Whole Blood B Cell Positive Selection Kit Protocol

		EASYSEP™ MAGNET
STEP	INSTRUCTIONS	**"The Big Easy" (Catalog #18001)** 
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.**	25 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.**	25 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 mL • Top up to 10 mL for samples > 2 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. Mix by gently pipetting up and down 2 - 3 times.**	Add 10 mL
	Replace the tube (without lid) into the magnet and incubate.	RT for 5 minutes
9	**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
10	**Repeat steps as indicated.**	Steps 8 and 9 (for a total of 1 x 10-minute and 2 x 5-minute separations)
11	**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™ HLA Whole Blood B Cell 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 B Cell WB Positive Selection 18184HLA
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 CD19+ cells by flow cytometry use one of the following fluorochrome-conjugated antibody clones:
- Anti-Human CD19 Antibody, Clone HIB19 (Catalog #60005), 4G7, or FMV63 (all partially blocked)

One of the following methods can also be used:
- Use an alternative marker such as fluorochrome-conjugated Anti-Human CD22 Antibody, Clone HIB22 (Catalog #60083) to detect CD22+ cells.
- Use alternative markers such as fluorochrome-conjugated Anti-Human CD45 Antibody, Clone HI30 (Catalog #60018) and Anti-Human CD43 Antibody, Clone CD43-10G7 (Catalog #60085) to detect CD45+CD43- cells.
- Use a fluorochrome-conjugated secondary antibody, such as Goat Anti-Mouse IgG (H+L) Antibody, Polyclonal (Catalog #60138).

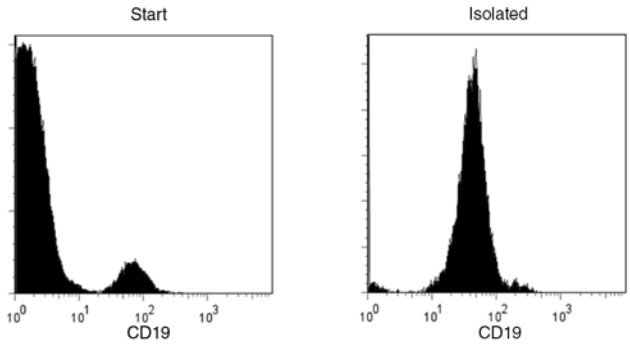
DONOR VARIABILITY

Certain donors express one or more soluble serum factors that can cause cross-linking with magnetic particles. This may result in visible aggregates in the enriched cell fraction following positive selection. These aggregates may appear as a distinct, high side-scatter population on FSC vs. SSC plots during flow cytometry analysis of the enriched fraction. This population consists solely of particles, with no cells or platelets present, as determined by staining with fluorescently-labeled antibodies against dextran, CD41, and CD45.

Potential aggregation can be avoided by washing away the donor plasma. Dilute the sample 2-fold in the recommended medium, and centrifuge at 300 x g for 10 minutes. Remove as much plasma as possible without disturbing the white and red blood cells, then resuspend the sample to the original volume with recommended medium before beginning the separation procedure.

If the samples have not been washed, any aggregates can be gated out during flow cytometry analysis of the enriched fraction based on their FSC vs. SSC characteristics, or by their lack of CD45 expression.

Data



Starting with fresh whole blood, the B cell content of the isolated fraction typically ranges from 86 - 97% (gated on CD45+ cells). In the above example, the purities of the start and final isolated fractions are 7% and 92%, respectively.

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