EasySep™ Serology Whole Blood CD19 Positive Selection Kit

For processing 60 mL of whole blood

Catalog #18984

Positive Selection

Document #10000005215 | Version 01

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Description

Isolate highly purified CD19+ cells from fresh human whole blood by immunomagnetic positive selection.

- Fast and easy-to-use
- Up to 98% purity
- No columns required
- Compatible with HLA serology

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 HLA serology, flow cytometry, or DNA/RNA extraction.

Component Descriptions

COMPONENT NAME	COMPONENT #	QUANTITY	STORAGE	SHELF LIFE	FORMAT
Anti-Human CD19 Antibody, Biotin	18984B	1 x 80 µL	Store at 2 - 8°C. Do not freeze.	Stable until expiry date (EXP) on label.	A combination of monoclonal antibodies in PBS and 0.09% sodium azide.
EasySep™ Serology Whole Blood CD19 Positive Selection Cocktail	18984C	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 and 0.1% BSA. Includes an Fc receptor blocking antibody.
EasySep™ Streptavidin RapidSpheres™ 50003	50003	2 x 1 mL	Store at 2 - 8°C. Do not freeze.	Stable until expiry date (EXP) on label.	A suspension of magnetic particles in PBS.
EasySep™ Serology Whole Blood CD19 Dissociation Reagent	18984D	3 x 0.5 mL	Store at 2 - 8°C. Do not freeze.	Stable until expiry date (EXP) on label.	A reagent diluted in PBS and 0.09% sodium azide.
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.

BSA - bovine serum albumin; 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
Isolation Cocktail (combined 18984B + 18984C)	Store at 2 - 8°C. Do not freeze.	Stable for up to 2 weeks. Do not exceed the expiry date (EXP) of individual components.
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 the original component.

Sample Preparation

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. Media should be free of Ca++ and Mg++.

Directions for Use – Manual EasySep™ Protocol

See page 1 for Sample Preparation and Recommended Medium. Refer to Table 1 for detailed instructions regarding the EasySep™ procedure.

Table 1. EasySep™ Serology Whole Blood CD19 Positive Selection Kit Protocol

		EASYSEP™ MAGNET
STEP	INSTRUCTIONS	**"The Big Easy" (Catalog #18001)** 
1	Add whole blood to required tube.	1 - 4.5 mL
	Required tube.	14 mL (17 x 95 mm) polystyrene round-bottom tube (e.g. Catalog #38008)
2	Add 1X EasySep™ RBC Lysis Buffer to sample.	Equal volume to sample
3	Prepare Isolation Cocktail.	Add 25 µL of Anti-Human CD19 Antibody, Biotin (18984B) to an entire vial of CD19 Positive Selection Cocktail (18984C). Isolation Cocktail is stable at 2 - 8°C for up to 2 weeks.
4	Add Isolation Cocktail to sample.	25 µL/mL of diluted sample
	Mix and incubate.	RT for 15 minutes
5	Vortex RapidSpheres™. NOTE: Particles should appear evenly dispersed.	30 seconds
6	Add RapidSpheres™ to sample.	10 µL/mL of diluted sample
	Mix and incubate.	RT for 10 minutes
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 10 mL
	Place the tube (without lid) into the magnet and incubate.	RT for 10 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
9	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 10 mL
	Place the tube (without lid) into the magnet and incubate.	RT for 5 minutes
10	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
11	Repeat steps as indicated.	Steps 9 and 10 (total of 1 x 10-minute and 2 x 5-minute separations)
12	Resuspend cells in desired medium. Be sure to collect cells from the sides of the tube.	0.5 - 1 mL
13	Vortex Dissociation Reagent.	30 seconds
14	Add Dissociation Reagent to resuspended cells.	20 µL/mL of resuspended cells
		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™ Serology Whole Blood CD19 Positive Selection Kit Protocol

STEP	INSTRUCTIONS	RoboSep™ (Catalog #20000 and #21000) 
1	Add whole blood to required tube.	1 - 4.5 mL
	Required tube.	14 mL (17 x 95 mm) polystyrene round-bottom tube (e.g. Catalog #38008)
2	Add 1X EasySep™ RBC Lysis Buffer to sample.	Equal volume to sample
3	Prepare Isolation Cocktail.	Add 25 µL of Anti-Human CD19 Antibody, Biotin (18984B) to an entire vial of CD19 Positive Selection Cocktail (18984C). Isolation Cocktail is stable at 2 - 8°C for up to 2 weeks.
4	Select protocol. NOTE: Enter volume.	Serology Whole Blood CD19 Positive Selection 18984 NOTE: Enter diluted sample volume.
5	Vortex RapidSpheres™. NOTE: Particles should appear evenly dispersed.	30 seconds
6	Load the carousel.	Follow on-screen prompts
	Start the protocol.	Press the green “Run” button
7	Unload the carousel when the run is complete. Remove the tube containing the isolated cells from the magnet and resuspend in desired medium. Be sure to collect cells from the sides of the tube.	0.5 - 1 mL
8	Vortex Dissociation Reagent.	30 seconds
9	Add Dissociation Reagent to resuspended cells.	20 µL/mL of resuspended cells
		Isolated cells are ready for use

Notes and Tips

EASYSEP™ RBC 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 by flow cytometry, use one of the following fluorochrome-conjugated antibody clones:

- Anti-Human CD19 Antibody, Clone HIB19 (Catalog #60005; partial blocking), or
- Anti-human CD19 antibody, clone 4G7 or FMV63 (partial blocking)

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 CD19-positive purity by up to 15%.
- Use a fluorochrome-conjugated secondary antibody, such as Goat Anti-Mouse IgG (H+L) Antibody, Polyclonal (Catalog #60138).

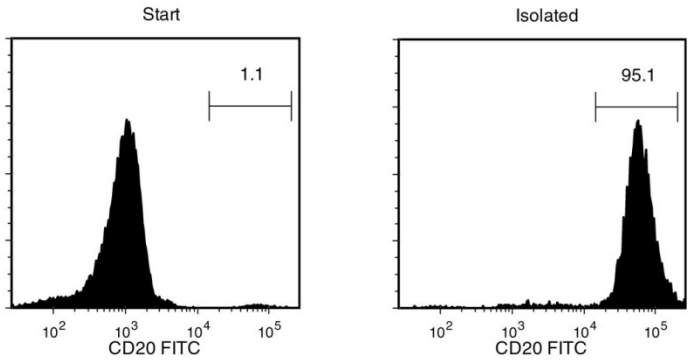
DONOR VARIABILITY

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 human whole blood, the CD19+ cell content of the isolated fraction typically ranges from 89.4 - 97.8%. In the above example, the purities of the start and final isolated fractions are 1.1% and 95.1%, respectively.

NOTE: Red blood cells were removed from start sample by lysis prior to flow cytometry.