



**EasySep™ Serology Whole Blood  
CD19 Positive Selection Kit**

Positive Selection

Catalog #18984

For processing 60 mL whole blood



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TOLL FREE PHONE 1 800 667 0322 • PHONE +1 604 877 0713

[INFO@STEMCELL.COM](mailto:INFO@STEMCELL.COM) • [TECHSUPPORT@STEMCELL.COM](mailto:TECHSUPPORT@STEMCELL.COM)

FOR GLOBAL CONTACT DETAILS VISIT OUR WEBSITE

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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
EasySep™ Serology Whole Blood CD19 Positive Selection Cocktail	18984C.1	2 x 0.65 mL	Store at 2 - 8°C. Do not freeze.	Stable until expiry date (EXP) on label.	A combination of monoclonal antibodies in PBS and 2% HPCD. Includes an Fc receptor blocking antibody.
EasySep™ Releasable RapidSpheres™ 50201	50201	4 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.

HPCD - 2-hydroxypropyl-β-cyclodextrin; PBS - phosphate-buffered saline

Components may be shipped at room temperature (15 - 25°C) but should be stored as indicated above.

RoboSep™ Serology Whole Blood CD19 Positive Selection Kit (18984RF) includes EasySep™ EasyTube™-14 (Catalog #20128) for optimal performance. The use of EasySep™ EasyTube™-14 is not required when performing a manual separation.

## 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 and 2 for detailed instructions regarding the EasySep™ procedure for each magnet.


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

		EASYSEP™ MAGNET
STEP	INSTRUCTIONS	<b>“The Big Easy” (Catalog #18001)</b> 
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 PBS to sample.	Equal volume to sample
3	Add Isolation Cocktail to sample.	10 µL/mL of diluted sample
	Mix and incubate.	RT for 5 minutes
4	Vortex RapidSpheres™. NOTE: Particles should appear evenly dispersed.	30 seconds
5	Add RapidSpheres™ to sample.	25 µL/mL of diluted sample
	Mix and incubate.	RT for 5 minutes
6	Add recommended medium to top up the sample to the indicated volume. Mix by gently pipetting up and down 2 - 3 times.	<ul style="list-style-type: none"> <li>• Top up to 5 mL for samples &lt; 4 mL</li> <li>• Top up to 10 mL for samples ≥ 4 mL</li> </ul>
	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 to the indicated volume. Mix by gently pipetting up and down 2 - 3 times.	<ul style="list-style-type: none"> <li>• Top up to 5 mL for samples &lt; 4 mL</li> <li>• Top up to 10 mL for samples ≥ 4 mL</li> </ul>
	Place 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 (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); PBS – phosphate-buffered saline

\* 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.

**Table 2. EasySep™ Serology Whole Blood CD19 Positive Selection Kit Protocol**

		EASYSEP™ MAGNET	
STEP	INSTRUCTIONS	EasyEights™ (Catalog #18103)	
		14 mL tube	
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 PBS to sample.	Equal volume to sample	
3	Add Isolation Cocktail to sample.	10 µL/mL of diluted sample	
	Mix and incubate.	RT for 5 minutes	
4	Vortex RapidSpheres™. NOTE: Particles should appear evenly dispersed.	30 seconds	
5	Add RapidSpheres™ to sample.	25 µL/mL of diluted sample	
	Mix and incubate.	RT for 5 minutes	
6	Add recommended medium to top up the sample to the indicated volume. Mix by gently pipetting up and down 2 - 3 times.	<ul style="list-style-type: none"> <li>• Top up to 5 mL for samples &lt; 4 mL</li> <li>• Top up to 10 mL for samples ≥ 4 mL</li> </ul>	
	Place the tube (without lid) into the magnet and incubate.	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	
8	Add recommended medium to top up the sample to the indicated volume. Mix by gently pipetting up and down 2 - 3 times.	<ul style="list-style-type: none"> <li>• Top up to 5 mL for samples &lt; 4 mL</li> <li>• Top up to 10 mL for samples ≥ 4 mL</li> </ul>	
	Place the tube (without lid) into the magnet and incubate.	RT for 5 minutes	
9	Carefully pipette** (do not pour) 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 (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); PBS – phosphate-buffered saline

\*\* Collect the entire supernatant, all at once, into a single pipette (e.g. for EasyEights™ 14 mL tube use a 10 mL serological pipette [Catalog #38004]).

## 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™ 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 PBS to sample.	Equal volume to sample	
3	Select protocol. NOTE: Enter volume.	HLA Serology Whole Blood CD19 Positive Selection 18984 v2 NOTE: Enter diluted sample volume.	
4	Vortex RapidSpheres™. NOTE: Particles should appear evenly dispersed.	30 seconds	
5	Load the carousel.	Follow on-screen prompts NOTE: When prompted to load a Separation Tube, place EasySep™ EasyTube™-14 into the magnet.	
	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.	Isolated cells are ready for use	

PBS – phosphate-buffered saline

## Notes and Tips

### 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).

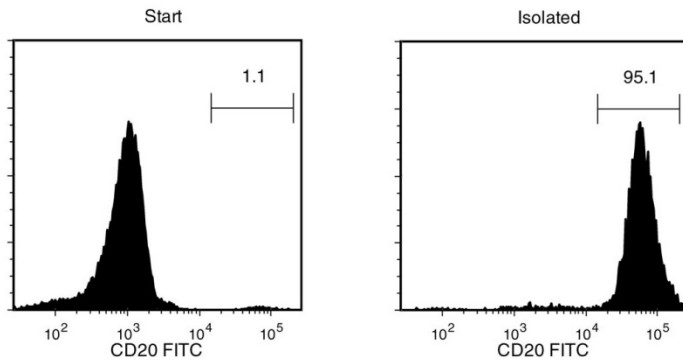
### DONOR VARIABILITY

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.

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 is typically  $93.6 \pm 6.2\%$  (mean  $\pm$  SD using “The Big Easy” EasySep™ Magnet). 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.

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