EasySep™ Direct HLA B Cell Isolation Kit

For processing 100 mL whole blood

Catalog #89684

Negative Selection

Document #10000003331 | Version 03

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ENGLISH

Intended Use

For the isolation of B cells from human whole blood, spleen, or lymph node samples. For in vitro diagnostic use.

Product Description

This kit targets non-B cells for removal by immunomagnetic negative selection with antibodies recognizing specific cell surface markers. Unwanted cells are labeled with antibodies and EasySep™ Direct RapidSpheres™, and separated using an EasySep™ magnet. Desired cells are simply collected into a new tube and are immediately available for downstream in vitro diagnostic applications.

Quality Control

EasySep™ Direct products are manufactured using aseptic technique and tightly controlled processes.

Each lot of EasySep™ Direct cocktail and particles is sterility tested according to USP methods and performance tested in cell isolation assays.

Storage and Stability

Store at 2 - 8°C. This product may be shipped at 15 - 25°C, but should be refrigerated upon receipt. Do not freeze. Product stable at 2 - 8°C until expiry date (Use-by date) on label.

Warnings and Precautions

1. For in vitro diagnostic use by laboratory professionals.
2. Do not use if vial contents have leaked. Unused product may be disposed of according to standard laboratory procedures for non-hazardous liquids.
3. This product should be handled by trained personnel observing good laboratory practices. Once this product is added to human cells, treat the suspension as potentially biohazardous. Handling of reagents and disposal of wastes should observe all local, state, or national regulations.
4. This product is a potential irritant to eyes, respiratory system, and skin. This product may also be harmful if ingested. Avoid exposure through skin, eye contact, inhalation, and ingestion.
5. RoboSep™ is provided as a piece of general laboratory equipment. User is responsible for validating RoboSep™ to meet their specific requirements.
6. A low starting cell frequency may lead to variability in purity of isolated B cells. End users may assess the purity of B cells after isolation by immunotyping using flow cytometry. End users may complete a cell count or equivalent test to confirm the number of B cells.
7. Users should follow all protocol steps in Directions for Use. Improper execution of protocol may lead to variable and/or poor results.
8. Users are responsible for validating the performance of downstream assays carried out using enriched B cells.

Component Descriptions

COMPONENT NAME	COMPONENT #	QUANTITY	FORMAT
EasySep™ Direct HLA B Cell Isolation Cocktail	89684C	2 x 2.5 mL	A combination of monoclonal antibodies in PBS.
EasySep™ Direct RapidSpheres™ 50302	50302	4 x 2.5 mL	A suspension of magnetic particles and monoclonal antibodies in PBS.

PBS - phosphate-buffered saline

Precipitate may be observed in the cocktail vial but will not affect performance.

Sample Preparation

PERIPHERAL BLOOD

For optimal red blood cell (RBC) depletion, collect blood using heparin or acid citrate dextrose (ACD) as an anticoagulant.
For best recovery, use unprocessed human whole blood. Recovery of the desired enriched cells decreases with samples that are older than 24 hours.
The volume of sample that can be processed depends on the EasySep™ magnet used for the enrichment procedure. Samples must be placed in the required tube to properly fit into the appropriate EasySep™ magnet (see Tables 1 - 2).

SPLEEN or LYMPH NODE

Disrupt spleen or lymph node in PBS or Hanks' Balanced Salt Solution (HBSS) containing 2% fetal bovine serum (FBS). Remove aggregates and debris by passing the cell suspension through a pre-wetted 100 µm mesh nylon strainer. Rinse the strainer with PBS or HBSS containing 2% FBS. Centrifuge at 300 x g for 10 minutes and resuspend at 1 - 100 x 10^6 cells/mL in recommended medium.

Recommended Medium

D-PBS (Without Ca++ and Mg++; Catalog #37350)

Materials Required But Not Provided

EasySep™ Magnet (Catalog #18000), “The Big Easy” EasySep™ Magnet (Catalog #18001), Easy 50 EasySep™ Magnet (Catalog #18002), EasyEights™ EasySep™ Magnet (Catalog #18103), or RoboSep™-S (Catalog #21000).

Directions for Use – Manual EasySep™ Protocols

See page 2 for Sample Preparation and Recommended Medium. Refer to Tables 1 - 4 for detailed instructions regarding the EasySep™ procedure for each magnet.

Table 1. EasySep™ Direct HLA B Cell Isolation Kit Protocol for WHOLE BLOOD

		EASYSEP™ MAGNETS	
STEP	INSTRUCTIONS	 **EasySep™ (Catalog #18000)**	**"The Big Easy" (Catalog #18001)** 
1	Collect sample within the volume range.	0.5 - 1.5 mL	1 - 7 mL
	Add sample to required tube.	5 mL (12 x 75 mm) polystyrene round-bottom tube (e.g. Catalog #38007)	14 mL (17 x 95 mm) polystyrene round-bottom tube (e.g. Catalog #38008)
2	Vortex RapidSpheres™. NOTE: Particles should appear evenly dispersed.	30 seconds	30 seconds
3	Add Isolation Cocktail to sample.	50 µL/mL of sample	50 µL/mL of sample
4	Add RapidSpheres™ to sample.	50 µL/mL of sample	50 µL/mL of sample
	Mix and incubate.	RT for 5 minutes	RT for 5 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 double the volume for samples ≤ 5 mL • Top up to 10 mL for samples > 5 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 the enriched cell suspension* into a new tube.	Use a new 5 mL tube	Use a new 14 mL tube
7	Add RapidSpheres™ to the new tube containing the enriched cells.	Use same volume as in step 4	Use same volume as in step 4
	Mix and incubate.	RT for 5 minutes	RT for 5 minutes
8	Remove the tube from the magnet; place the tube from step 7 (without lid) into the magnet and incubate for a second separation.	RT for 5 minutes	RT for 5 minutes
9	Pick up the magnet, and in one continuous motion invert the magnet and tube,** pouring the enriched cell suspension into a new tube.	Isolated cells are ready for use	Use a new 14 mL tube
10	Remove the tube from the magnet; place the new tube from step 9 (without lid) into the magnet and incubate for a third separation.	---	RT for 5 minutes
11	Pick up the magnet, and in one continuous motion invert the magnet and tube,** pouring the enriched cell suspension into a new tube.	---	Isolated cells are ready for use

RT - room temperature (15 - 25°C)
* Following the first magnetic separation the collected cells may contain a significant amount of RBCs and may look similar to the original unprocessed human whole blood sample.
** To minimize RBC contamination in the isolated cells, pour off the sample along a clean area of the tube (i.e. the opposite side to where the sample was poured in).

Table 2. EasySep™ Direct HLA B Cell Isolation Kit Protocol for WHOLE BLOOD

		EASYSEP™ MAGNETS		
		EasyEights™ (Catalog #18103)		
STEP	INSTRUCTIONS	5 mL tube	14 mL tube	Easy 50 (Catalog #18002)
1	Collect sample within the volume range.	0.5 - 1.5 mL	1 - 7 mL	7 - 30 mL
	Add sample to required tube.	5 mL (12 x 75 mm) polystyrene round-bottom tube (e.g. Catalog #38007)	14 mL (17 x 95 mm) polystyrene round-bottom tube (e.g. Catalog #38008)	50 mL conical tube (e.g. Catalog #38010)
2	Vortex RapidSpheres™. NOTE: Particles should appear evenly dispersed.	30 seconds	30 seconds	30 seconds
3	Add Isolation Cocktail to sample.	50 µL/mL of sample	50 µL/mL of sample	50 µL/mL of sample
4	Add RapidSpheres™ to sample.	50 µL/mL of sample	50 µL/mL of sample	50 µL/mL of sample
	Mix and incubate.	RT for 5 minutes	RT for 5 minutes	RT for 5 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 double the volume for samples ≤ 5 mL • Top up to 10 mL for samples > 5 mL	• Top up to double the volume for samples ≤ 25 mL • Top up to 50 mL for samples > 25 mL
	Place the tube (without lid) into the magnet and incubate.	RT for 5 minutes	RT for 5 minutes	RT for 10 minutes
6	Carefully pipette*** (do not pour) the enriched cell suspension into a new tube. NOTE: Collect the entire clear fraction from top to bottom. For optimal recovery, also collect a small volume of RBCs (up to 10% of the starting sample volume).	Use a new 5 mL tube	Use a new 14 mL tube	Use a new 50 mL tube
7	Add RapidSpheres™ to the new tube containing the enriched cells.	Use same volume as in step 4	Use same volume as in step 4	Use same volume as in step 4
	Mix and incubate.	RT for 5 minutes	RT for 5 minutes	RT for 5 minutes
8	Remove the tube from the magnet; place the tube from step 7 (without lid) into the magnet and incubate for a second separation.	RT for 5 minutes	RT for 5 minutes	RT for 5 minutes
9	Carefully pipette*** (do not pour) the enriched cell suspension into a new tube. NOTE: Collect only the clear fraction.	Use a new 5 mL tube	Use a new 14 mL tube	Use a new 50 mL tube
10	Remove the tube from the magnet; place the new tube from step 9 (without lid) containing the enriched cells into the magnet and incubate for a third separation.	RT for 5 minutes	RT for 5 minutes	RT for 5 minutes
11	Carefully pipette*** (do not pour) the enriched cell suspension into a new tube. NOTE: Collect only the clear fraction.	Isolated cells are ready for use	Isolated cells are ready for use	Isolated cells are ready for use

RT - room temperature (15 - 25°C)

*** Collect the entire supernatant, all at once, into a single pipette (e.g. for EasyEights™ 5 mL tube use a 2 mL serological pipette [Catalog #38002]; for EasyEights™ 14 mL tube use a 10 mL serological pipette [Catalog #38004]).

Table 3. EasySep™ Direct HLA B Cell Isolation Kit Protocol for SPLEEN or LYMPH NODE

		EASYSEP™ MAGNETS	
STEP	INSTRUCTIONS	 **EasySep™ (Catalog #18000)**	**"The Big Easy" (Catalog #18001)** 
1	Collect sample within the volume range.	0.5 - 1.5 mL	1 - 7 mL
	Add sample to required tube.	5 mL (12 x 75 mm) polystyrene round-bottom tube (e.g. Catalog #38007)	14 mL (17 x 95 mm) polystyrene round-bottom tube (e.g. Catalog #38008)
2	Add Isolation Cocktail to sample.	50 µL/mL of sample	50 µL/mL of sample
	Mix and incubate.	RT for 5 minutes	RT for 5 minutes
3	Vortex RapidSpheres™. NOTE: Particles should appear evenly dispersed.	30 seconds	30 seconds
4	Add RapidSpheres™ to sample. Mix by gently pipetting up and down 2 - 3 times.	50 µL/mL of sample	50 µL/mL of sample
5	Add recommended medium to top up the sample to the indicated volume.	Top up to 2.5 mL	• Top up to double the volume for samples ≤ 5 mL • Top up to 10 mL for samples > 5 mL
	Place the tube (without lid) into the magnet and incubate.	RT for 3 minutes	RT for 3 minutes
6	Pick up the magnet, and in one continuous motion invert the magnet and tube, pouring the enriched cell suspension into a new tube.	Use a new 5 mL tube	Use a new 14 mL tube
7	Add RapidSpheres™ to the new tube containing the enriched cells. Mix by gently pipetting up and down 2 - 3 times.	Use same volume as in step 4	Use same volume as in step 4
8	Remove the tube from the magnet and place the tube from step 7 (without lid) into the magnet and incubate for a second separation.	RT for 3 minutes	RT for 3 minutes
9	Pick up the magnet, and in one continuous motion invert the magnet and tube, pouring the enriched cell suspension into a new tube.	Isolated cells are ready for use	Isolated cells are ready for use

RT - room temperature (15 - 25°C)

Table 4. EasySep™ Direct HLA B Cell Isolation Kit Protocol for SPLEEN or LYMPH NODE

		EASYSEP™ MAGNETS	
		EasyEights™ (Catalog #18103)	
STEP	INSTRUCTIONS	 5 mL tube	14 mL tube 
1	Collect sample within the volume range.	0.5 - 1.5 mL	1 - 7 mL
	Add sample to required tube.	5 mL (12 x 75 mm) polystyrene round-bottom tube (e.g. Catalog #38007)	14 mL (17 x 95 mm) polystyrene round-bottom tube (e.g. Catalog #38008)
2	Add Isolation Cocktail to sample.	50 µL/mL of sample	50 µL/mL of sample
	Mix and incubate.	RT for 5 minutes	RT for 5 minutes
3	Vortex RapidSpheres™. NOTE: Particles should appear evenly dispersed.	30 seconds	30 seconds
4	Add RapidSpheres™ to sample. Mix by gently pipetting up and down 2 - 3 times.	50 µL/mL of sample	50 µL/mL of sample
5	Add recommended medium to top up sample to the indicated volume. Mix by gently pipetting up and down 2 - 3 times.	Top up to 2.5 mL	• Top up to double the volume for samples ≤ 5 mL • Top up to 10 mL for samples > 5 mL
	Place the tube (without lid) into the magnet and incubate.	RT for 3 minutes	RT for 3 minutes
6	Carefully pipette*** (do not pour) the enriched cell suspension into a new tube. NOTE: Collect the entire clear fraction from top to bottom. For optimal recovery, also collect a small volume of RBCs, if present (up to 10% of the starting sample volume).	Use a new 5 mL tube	Use a new 14 mL tube
7	Add RapidSpheres™ to the new tube containing the enriched cells. Mix by gently pipetting up and down 2 - 3 times.	Use same volume as in step 4	Use same volume as in step 4
8	Remove the tube from the magnet; place the tube from step 7 (without lid) into the magnet and incubate for a second separation.	RT for 3 minutes	RT for 3 minutes
9	Carefully pipette*** (do not pour) the enriched cell suspension into a new tube. NOTE: Collect only the clear fraction.	Use a new 5 mL tube **For lymph node**: Isolated cells are ready for use	Use a new 14 mL tube **For lymph node**: Isolated cells are ready for use
10	Remove the tube from the magnet; place the new tube from step 9 (without lid) containing the enriched cells into the magnet and incubate for a third separation.	**For spleen:** RT for 3 minutes	**For spleen**: RT for 3 minutes
11	Carefully pipette*** (do not pour) the enriched cell suspension into a new tube. NOTE: Collect only the clear fraction.	**For spleen**: Isolated cells are ready for use	**For spleen**: Isolated cells are ready for use

RT - room temperature (15 - 25°C)

*** Collect the entire supernatant, all at once, into a single pipette (e.g. for EasyEights™ 5 mL tube use a 2 mL serological pipette [Catalog #38002]; for EasyEights™ 14 mL tube use a 10 mL serological pipette [Catalog #38004]).

Directions for Use – Fully Automated RoboSep™ Protocol

See page 2 for Sample Preparation and Recommended Medium. Refer to Table 5 for detailed instructions regarding the RoboSep™ procedure.
NOTE: If using RoboSep™-S, ensure the software is at least v.1.2.0.2 and RoboSep™ Direct-compatible carousel is installed. Contact us at techsupport@stemcell.com for more information.

Table 5. RoboSep™ Direct HLA B Cell Isolation Kit Protocol for WHOLE BLOOD, SPLEEN, or LYMPH NODE

STEP	INSTRUCTIONS	RoboSep™ (Catalog #20000 and #21000) 
1	Prepare sample at the indicated cell concentration within the volume range.	**For spleen or lymph node:** 1 - 6 mL at 1 - 100 x 10^6 cells/mL **For blood:** 1 - 6 mL
	Add sample to required tube.	14 mL (17 x 95 mm) polystyrene round-bottom tube (e.g. Catalog #38008)
2	Select protocol.	EasySep Direct HLA B Cell Isolation 89684 - For WB, Spleen, LN
3	Vortex RapidSpheres™. NOTE: Particles should appear evenly dispersed.	30 seconds
4	Load the carousel.	Follow on-screen prompts NOTE: This protocol requires loading **two** vials of EasySep™ Direct RapidSpheres™ 50300 onto the carousel for a single run; one in the ▲ (triangle) slot and one in the ● (circle) slot.
	Start the protocol.	Press the green “Run” button
5	Unload the carousel when the run is complete.	Isolated cells are ready for use

Notes and Tips

REMOVAL OF RESIDUAL RBCs IN THE ISOLATED CELLS

Typically, further RBC depletion is not required following cell isolation. If residual RBCs are visible in the isolated cell pellet following centrifugation after the end of the protocol, resuspend in a small volume (0.2 - 2.5 mL) of recommended medium or desired culture medium and place in a smaller EasySep™ magnet for an additional 5-minute separation. Collect the supernatant and the isolated cells are now ready for use in downstream applications. Residual RBCs may also be lysed using Ammonium Chloride Solution (Catalog #07800).

ASSESSING PURITY

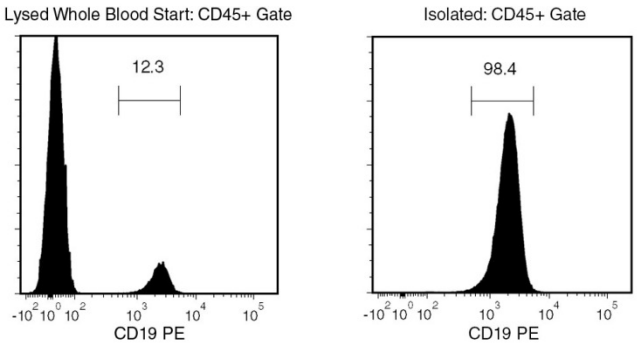
For purity assessment of B cells by flow cytometry, use the following fluorochrome-conjugated antibody clones:

- Anti-Human CD19 Antibody, Clone HIB19 (Catalog #60005), and
- Anti-Human CD45 Antibody, Clone HI30 (Catalog #60018)

NOTE: It is recommended to assess purity on CD45+ cells to exclude debris, platelets, and RBCs. Include a viability dye if necessary (e.g. Propidium Iodide [Catalog #75002]; 7-AAD [7- Aminoactinomycin D; Catalog #75001]).

Data

Starting with human whole blood from normal healthy donors, the B cell (CD19+) content of the non-lysed final isolated fraction typically ranges from 89 - 99.8% (gated on CD45).



In the above example, the B cell (CD19+) content of the lysed whole blood start sample and non-lysed final isolated fraction is 12.3% and 98.4% (gated on CD45), respectively.

Technical Assistance

For technical support, contact us at techsupport@stemcell.com or call either +1.604.877.0713 or the European toll-free number 00800 7836 2355. For more information, visit www.stemcell.com.

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