EasySep™ Direct Human PBMC Isolation Kit

For processing 100 mL whole blood

Catalog #19654

Negative Selection

Document #10000003347 | Version 04

STEMCELL™ TECHNOLOGIES
Scientists Helping Scientists™ | WWW.STEMCELL.COM
TOLL FREE PHONE 1 800 667 0322 • PHONE +1 604 877 0713
INFO@STEMCELL.COM • TECHSUPPORT@STEMCELL.COM
FOR GLOBAL CONTACT DETAILS VISIT OUR WEBSITE

Description

Isolate highly purified peripheral blood mononuclear cells (PBMCs) directly from human whole blood by immunomagnetic negative selection. This kit can also be used to isolate PBMCs from other sample types (see Table 1).

- 99.9% RBC depletion without the need for density gradient centrifugation, sedimentation, or lysis
- Fast, easy-to-use, and column-free
- Isolated cells are untouched

This kit targets granulocytes, platelets, and red blood cells (RBCs) for removal with antibodies recognizing specific cell surface markers. Unwanted cells are labeled with antibodies and magnetic particles and separated using an EasySep™ magnet. PBMCs are simply collected into a new tube and are immediately available for downstream applications, such as flow cytometry, culture, or DNA/RNA extraction.

NOTE: This is the Product Information Sheet (PIS) for isolating PBMCs from whole blood. If isolating PBMCs from other sample types, refer to the applicable PIS Document Number (see Table 1), available at www.stemcell.com or contact us to request a copy.

Table 1. Applicable PIS Document Number for Other Sample Types

SAMPLE TYPE	PIS DOCUMENT NUMBER
Buffy coat	10000012559
Bone marrow	10000012544
Cord blood	10000012560
Leukapheresis	10000012561
Leukoreduction system chamber (LRSC)	10000012562

Component Descriptions

COMPONENT NAME	COMPONENT #	QUANTITY	STORAGE	SHELF LIFE	FORMAT
EasySep™ Direct Human PBMC Isolation Cocktail	19654C	2 x 2.5 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™ Direct RapidSpheres™ 50300	50300	4 x 2.5 mL	Store at 2 - 8°C. Do not freeze.	Stable until expiry date (EXP) on label.	A suspension of magnetic particles and monoclonal antibodies in PBS.

PBS - phosphate-buffered saline

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

Sample Preparation

PERIPHERAL BLOOD

For best recovery, use unprocessed human whole blood. Recovery of the desired isolated cells decreases with samples that are older than 24 hours.

The volume of blood that can be processed depends on the EasySep™ magnet used for the isolation procedure. Blood samples must be placed in the required tube or flask to properly fit into the appropriate EasySep™ magnet.

To avoid loss of monocytes, EDTA must be added to the whole blood sample to a final concentration of 6 mM prior to labeling and separation (see step 2, Tables 2 - 5). An EDTA stock solution greater than 0.05 M is recommended to avoid overdiluting the start sample.

Recommended Medium

EasySep™ Buffer (Catalog #20144), RoboSep™ Buffer (Catalog #20104), D-PBS (Without Ca++ and Mg++; Catalog #37350), or PBS containing 2% 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 Tables 2 - 4 for detailed instructions regarding the EasySep™ procedure for each magnet.

Table 2. EasySep™ Direct Human PBMC Isolation Kit Protocol for WHOLE BLOOD

		EASYSEP™ MAGNETS	
STEP	INSTRUCTIONS	 **EasySep™ (Catalog #18000)**	**"The Big Easy" (Catalog #18001)** 
1	Prepare sample within the volume range.	1 - 2 mL	1 - 6 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 EDTA to sample.	At a final concentration of 6 mM EDTA	At a final concentration of 6 mM EDTA
3	Add Isolation Cocktail to sample. NOTE: Do not vortex cocktail.	50 µL/mL of sample	50 µL/mL of sample
	Mix and incubate.	RT for 5 minutes	RT for 5 minutes
4	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 double the original sample volume	Top up to double the original sample volume
5	Vortex RapidSpheres™. NOTE: Particles should appear evenly dispersed.	30 seconds	30 seconds
6	Add RapidSpheres™ to sample and mix.	50 µL/mL of original sample volume NOTE: No incubation, IMMEDIATELY proceed to next step	50 µL/mL of original sample volume NOTE: No incubation, IMMEDIATELY proceed to next step
7	Place the tube (without lid) into the magnet and incubate.	RT for 5 minutes	RT for 5 minutes
8	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
9	Add RapidSpheres™ to the new tube containing the enriched cells and mix.	Use same volume as in step 6 NOTE: No incubation, IMMEDIATELY proceed to next step	Use same volume as in step 6 NOTE: No incubation, IMMEDIATELY proceed to next step
10	Remove the tube from the magnet; place the tube from step 9 (without lid) into the magnet and incubate for a second separation.	RT for 5 minutes	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.	Use a new 5 mL tube	Use a new 14 mL tube
12	Remove the tube from the magnet; place the tube from step 11 (without lid) into the magnet and incubate for a third separation.	RT for 5 minutes	RT for 5 minutes
13	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)

* 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 3. EasySep™ Direct Human PBMC Isolation Kit Protocol for WHOLE BLOOD

		EASYSEP™ MAGNETS		
		EasyEights™ (Catalog #18103)		
STEP	INSTRUCTIONS	5 mL tube	14 mL tube	Easy 50 (Catalog #18002)
1	Prepare sample within the volume range.	1 - 2 mL	1 - 6 mL	5 - 25 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 (30 x 115 mm) conical tube (e.g. Catalog #38010)
2	Add EDTA to sample.	At a final concentration of 6 mM EDTA	At a final concentration of 6 mM EDTA	At a final concentration of 6 mM EDTA
3	Add Isolation Cocktail to sample. NOTE: Do not vortex cocktail.	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
4	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 double the original sample volume	Top up to double the original sample volume	• Top up to double the original sample volume for samples ≤ 20 mL • Top up to 50 mL for samples > 20 mL
5	Vortex RapidSpheres™. NOTE: Particles should appear evenly dispersed.	30 seconds	30 seconds	30 seconds
6	Add RapidSpheres™ to sample and mix.	50 µL/mL of original sample volume NOTE: No incubation, IMMEDIATELY proceed to next step	50 µL/mL of original sample volume NOTE: No incubation, IMMEDIATELY proceed to next step	50 µL/mL of original sample volume NOTE: No incubation, IMMEDIATELY proceed to next step
7	Place the tube (without lid) into the magnet and incubate.	RT for 5 minutes	RT for 5 minutes	RT for 10 minutes
8	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
9	Add RapidSpheres™ to the new tube containing the enriched cells and mix.	Use same volume as in step 6 NOTE: No incubation, IMMEDIATELY proceed to next step	Use same volume as in step 6 NOTE: No incubation, IMMEDIATELY proceed to next step	Use same volume as in step 6 NOTE: No incubation, IMMEDIATELY proceed to next step
10	Remove the tube from the magnet; place the tube from step 9 (without lid) into the magnet and incubate for a second separation.	RT for 5 minutes	RT for 5 minutes	RT for 10 minutes
11	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
12	Remove the tube from the magnet; place the new tube from step 11 (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 10 minutes
13	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 4. EasySep™ Direct Human PBMC Isolation Kit Protocol for WHOLE BLOOD

		EASYSEP™ MAGNETS
STEP	INSTRUCTIONS	**Easy 250 EasySep™ Magnet (Catalog #100-0821)** 
1	**Prepare sample within the volume range.**	25 - 125 mL
	Add sample to required flask.	T-75 cm^2 cell culture flask (i.e. Corning Catalog #353135)
2	**Add EDTA to sample.**	At a final concentration of 6 mM EDTA
3	**Add Isolation Cocktail to sample.** **NOTE: Do not vortex cocktail.**	50 µL/mL of sample
	Mix with a 25 mL or 50 mL serological pipette§ and incubate. **NOTE: Mixing can also be done by rotating or gently agitating the flask. Cap the flask first to prevent spillage.**	RT for 5 minutes
4	**Add recommended medium to top up the sample to the indicated volume.**	Top up to double the original sample volume
5	**Vortex RapidSpheres™.** **NOTE: Particles should appear evenly dispersed.**	30 seconds
6	**Add RapidSpheres™ to sample and mix as described in step 3.**	50 µL/mL of original sample volume NOTE: No incubation, IMMEDIATELY proceed to next step
7	**Place the flask (without cap) into the magnet and incubate.**	RT for 10 minutes
8	**Carefully pipette*** (do not pour) the cell suspension into a new flask.** **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 T-75 cm^2 flask
9	**Add RapidSpheres™ to the new flask containing the enriched cells and mix as described in step 3.**	Use same volume as in step 6 NOTE: No incubation, IMMEDIATELY proceed to next step
10	**Remove the flask from the magnet; place the flask from step 9 (without cap) into the magnet and incubate for a second separation.**	RT for 10 minutes
11	**Carefully pipette*** (do not pour) the enriched cell suspension into a new flask.**	Use a new T-75 cm^2 flask
12	**Remove the flask from the magnet; place the flask from step 11 (without cap) into the magnet and incubate for a third separation.**	RT for 10 minutes
13	**Carefully pipette*** (do not pour) the cell suspension into a new tube or centrifuge bottle.‡**	Isolated cells are ready for use

RT - room temperature (15 - 25°C)

§ e.g. 25 mL (Catalog #38005) or 50 mL (Catalog #38006) serological pipette

*** To collect the supernatant, gently sweep the pipette back and forth along the midline of the T-75 cm^2 flask while aspirating. Avoid touching the sides of the flask. Switch to a 10 mL or smaller serological pipette to collect the residual supernatant.

‡ e.g. 50 mL (30 x 115 mm) conical tube (Catalog #38010) or 225 mL centrifuge bottle (Corning Catalog #352075)

Directions for Use – Fully Automated RoboSep™ Protocol

See page 1 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 a carousel compatible with this product is installed. Contact us at techsupport@stemcell.com for more information.

Table 5. RoboSep™ Direct Human PBMC Isolation Kit Protocol for WHOLE BLOOD

STEP	INSTRUCTIONS	RoboSep™ (Catalog #21000) 
1	Prepare sample within the volume range.	1 - 6 mL
	Add sample to required tube.	14 mL (17 x 95 mm) polystyrene round-bottom tube (e.g. Catalog #38008)
2	Add EDTA to sample.	At a final concentration of 6 mM EDTA
3	Select protocol.	EasySep Direct Human PBMC Isolation 19654 - WB CB BM LEUK LRSC
4	Vortex RapidSpheres™. NOTE: Particles should appear evenly dispersed.	30 seconds
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.	Isolated cells are ready for use

Notes and Tips

If further downstream cell separation is required via magnetic positive selection products, contact us at techsupport@stemcell.com.

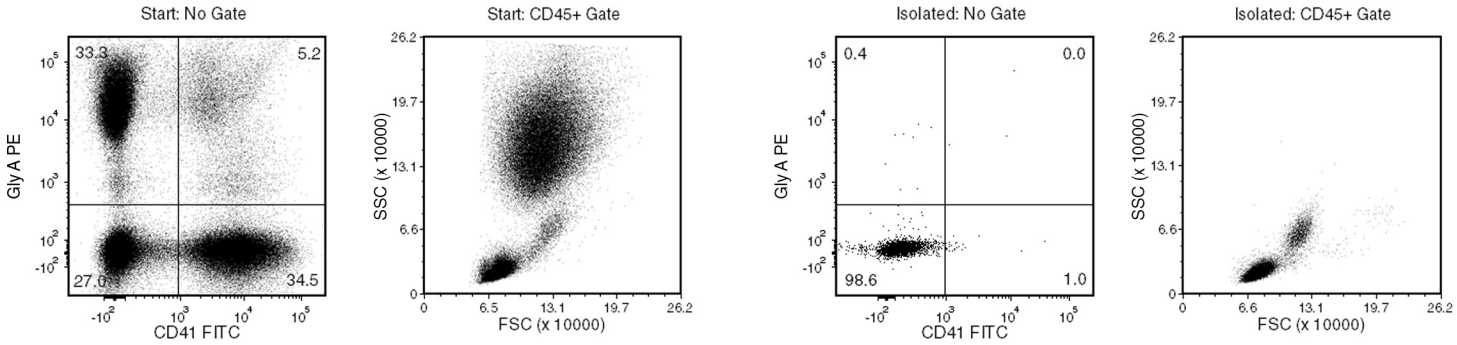
ASSESSING PURITY

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

- Anti-Human CD235ab (Glycophorin A/B) Antibody, Clone HIR2 (Catalog #60111), and
- Anti-Human CD41 Antibody, Clone HIP8 (Catalog #60114), and
- Anti-Human CD45 Antibody, Clone HI30 (Catalog #60018)

Data

Starting with human whole blood from normal healthy donors, the typical mononuclear cell content of the non-lysed final isolated fraction is 98.3 ± 2.8% (gated on CD45).



In the above example, the mononuclear cell content of the whole blood start sample (lysed by ammonium chloride) and non-lysed final isolated fraction is 27.0% and 98.6% (not gated on CD45), respectively.