EasySep™ Direct Human PBMC Isolation Kit

For processing 100 mL leukapheresis samples

Catalog #19654
#19654RF RoboSep™

Negative Selection

Document #10000012561 | Version 04

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Description

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

- 99.9% red blood cell (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 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 leukapheresis samples. 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
Whole blood	10000003347
Buffy coat	10000012559
Bone marrow	10000012544
Cord blood	10000012560
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

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

LEUKAPHERESIS SAMPLES

For best recovery, use unprocessed leukapheresis samples (e.g. Human Peripheral Blood Leukopak, Fresh, Catalog #70500*). Recovery of the desired isolated cells decreases with samples that are older than 24 hours.

To avoid the loss of monocytes, EDTA must be added to the leukapheresis 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 over-diluting the start sample.

* Some primary cell products are available only in select regions. Contact us at techsupport@stemcell.com for further information.

Recommended Medium

EasySep™ Buffer (Catalog #20144), RoboSep™ Buffer (Catalog #20104), D-PBS (Without Ca++ and Mg++; Catalog #37350), or PBS containing 2% fetal bovine serum 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 LEUKAPHERESIS SAMPLES

		EASYSEP™ MAGNETS	
STEP	INSTRUCTIONS	 **EasySep™ (Catalog #18000)**	**"The Big Easy" (Catalog #18001)** 
1	**Prepare sample within the volume range.**	0.5 - 1.5 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.**	Isolated cells are ready for use	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.

Table 3. EasySep™ Direct Human PBMC Isolation Kit Protocol for LEUKAPHERESIS SAMPLES

		EASYSEP™ MAGNETS		
STEP	INSTRUCTIONS	EasyEights™ (Catalog #18103)		Easy 50 (Catalog #18002)
		5 mL tube	14 mL tube	
1	Prepare sample within the volume range.	0.5 - 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 5 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 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 4. EasySep™ Direct Human PBMC Isolation Kit Protocol for LEUKAPHERESIS SAMPLES

		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 performed 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 5 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 5 minutes
11	**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 LEUKAPHERESIS SAMPLES

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.
- If performing an EDTA-sensitive downstream application, wash the isolated cell fraction in desired medium prior to use.

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)