EasySep™ Direct Human PBMC Isolation Kit

For processing 100 mL bone marrow

Catalog #19654

Negative Selection

Document #10000012544 | Version 01

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Description

Isolate highly purified peripheral blood mononuclear cells (PBMCs) directly from human bone marrow 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.

- This is the Product Information Sheet (PIS) for isolating PBMCs from bone marrow. If isolating PBMCs from other sample types, refer to the applicable PIS Document Number (see Table 1).

Table 1. Applicable Document Number for Other Sample Types

SAMPLE TYPE	DOCUMENT NUMBER
Whole blood	10000003347
Buffy coat	10000012559
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

BONE MARROW

1. Dilute the sample 5- to 10-fold in recommended medium and mix gently by pipetting up and down.
2. Centrifuge cells at 300 x g for 10 minutes with the brake on. Remove and discard the supernatant.
3. Resuspend the cell pellet with recommended medium. Filter the sample through a pre-wetted 70 µm strainer to remove bone fragments, cell aggregates, and debris. Rinse the strainer with recommended medium.
4. Centrifuge cells at 300 x g for 10 minutes with the brake off.
5. Carefully remove and discard the supernatant, without disturbing the cell pellet.
6. Resuspend the cell pellet to the original sample volume with recommended medium.

To avoid loss of monocytes, EDTA must be added to the bone marrow sample to a final concentration of 6 mM prior to labeling and separation (see step 2, Tables 2 - 4). 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 and 3 for detailed instructions regarding the EasySep™ procedure for each magnet.

Table 2. EasySep™ Direct Human PBMC Isolation Kit Protocol for BONE MARROW

		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.	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 human bone marrow 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 BONE MARROW

		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 - 20 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
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]).

Directions for Use – Fully Automated RoboSep™ Protocol

See page 1 for Sample Preparation and Recommended Medium. Refer to Table 4 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 4. RoboSep™ Direct Human PBMC Isolation Kit Protocol for BONE MARROW

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

- EasySep™ Direct Human PBMC Isolation Kit is not suitable for use with downstream magnetic positive selection.
- 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)