EasySep™

Positive Selection

Catalog #18352

EasySep™ Human Glycophorin A Depletion Kit

For processing 1.6 x 10^9 cells

STEMCELL™ TECHNOLOGIES

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Description

Deplete glycophorin A (GlyA)-postive cells from fresh or previously frozen human peripheral blood or bone marrow mononuclear cells (MNCs).

- Fast, easy-to-use, and column-free
- Isolated cells are untouched

This kit targets GlyA+ cells for removal with an antibody recognizing the GlyA surface marker. Unwanted cells are labeled with antibodies and magnetic particles, and separated without columns using an EasySep™ magnet. Desired cells are simply poured off into a new tube. Isolated cells are immediately available for downstream applications such as flow cytometry, culture, or DNA/RNA extraction.

Component Descriptions

COMPONENT NAME	COMPONENT #	QUANTITY	STORAGE	SHELF LIFE	FORMAT
EasySep™ Human GlyA Depletion Cocktail	18352C.2	1 x 1 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™ Magnetic Nanoparticles Positive Selection	18150	1 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.

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 and frozen samples, see www.stemcell.com/primarycells.

PERIPHERAL BLOOD or BONE MARROW

Prepare a MNC suspension from whole blood by centrifugation over a density gradient medium (e.g. Lymphoprep™, Catalog #07801). For more rapid MNC preparation, use the SepMate™ RUO (Catalog #86450/86415) or SepMate™ IVD* (Catalog #85450/85415) cell isolation tube.

If using previously frozen MNCs, incubate the cells with DNase I Solution (Catalog #07900) at a concentration of 100 µg/mL at room temperature (15 - 25°C) for at least 15 minutes prior to labeling and separation. Filter aggregated suspensions through a 40 µm Cell Strainer (Catalog #27305) for optimal results.

After preparation, resuspend cells at 2 x 10^8 cells/mL in recommended medium.

* SepMate™ IVD is only available in select regions where it is registered as an In Vitro Diagnostic (IVD) device for the isolation of MNCs from whole blood or bone marrow by density gradient centrifugation. In all other regions SepMate™ is available for research use only (RUO).

Recommended Medium

EasySep™ Buffer (Catalog #20144), RoboSep™ Buffer (Catalog #20104), or PBS containing 2% fetal bovine serum (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 Table 1 detailed instructions regarding the EasySep™ procedure for each magnet.

Table 1. EasySep™ Human Glycophorin A Depletion Kit Protocol

		EASYSEP™ MAGNETS	
STEP	INSTRUCTIONS	 **EasySep™ (Catalog #18000)**	**"The Big Easy" (Catalog #18001)** 
1	**Prepare sample at the indicated cell concentration within the volume range.**	2 x 10^8 cells/mL* 0.1 - 2 mL NOTE: If starting with fewer than 2 x 10^7 cells, resuspend cells in 0.1 mL	2 x 10^8 cells/mL* 0.25 - 8 mL NOTE: If starting with fewer than 5 x 10^7 cells, resuspend cells in 0.25 mL
	Add sample to required tube.	5 mL (12 x 75 mm) polystyrene round-bottom tube (e.g. Corning Catalog #352058)	14 mL (17 x 100 mm) polystyrene round-bottom tube (e.g. Corning Catalog #352057)
2	**Add Depletion Cocktail to sample.**	125 µL/mL of sample*	125 µL/mL of sample*
	Mix and incubate.	RT for 15 minutes	RT for 15 minutes
3	**Mix Magnetic Particles.** **NOTE: Particles should appear evenly dispersed.**	Pipette up and down more than 5 times	Pipette up and down more than 5 times
4	**Add Magnetic Particles to sample.**	125 µL/mL of sample*	125 µL/mL of sample*
	Mix and incubate.	RT for 10 minutes	RT for 10 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 5 mL for samples < 4.25 mL • Top up to 10 mL for samples ≥ 4.25 mL
	Place the tube (without lid) into the magnet and incubate.	RT for 10 minutes	RT for 10 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.**	Isolated cells are ready for use	Isolated cells are ready for use
OPTIONAL ADDITIONAL SEPARATION for PURITY **NOTE: This will improve depletion but may reduce recovery**		---	---
7	**Remove the tube from the magnet and place the new tube (without lid) into the magnet and incubate for a second separation.**	RT for 10 minutes	RT for 10 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.**	Isolated cells are ready for use	Isolated cells are ready for use

RT - room temperature (15 - 25°C)

* The appropriate cell concentration will vary depending on the application and it may be necessary to optimize the amount of cocktail and particles added in Steps 2 and 4.

** 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.

Directions for Use – Fully Automated RoboSep™ Protocol

See page 1 for Sample Preparation and Recommended Medium. Refer to Table 2 for detailed instructions regarding the RoboSep™ procedure.

Table 2. RoboSep™ Human Glycophorin A Depletion Kit Protocol

STEP	INSTRUCTIONS	RoboSep™ (Catalog #20000 and #21000) 
1	Prepare sample at the indicated cell concentration within the volume range.	2 x 10^8 cells/mL* 0.25 - 8 mL NOTE: If starting with fewer than 5 x 10^7 cells, resuspend cells in 0.25 mL
	Add sample to required tube.	14 mL (17 x 100 mm) polystyrene round-bottom tube (e.g. Corning Catalog #352057)
2	Select protocol.	Human GlyA Depletion 18352-high purity
3	Mix Magnetic Particles. NOTE: Particles should appear evenly dispersed.	Pipette up and down more than 5 times
4	Load the carousel.	Follow on-screen prompts
	Start the protocol.	Press the green “Run” button
5	Unload the carousel when the run is complete. Remove the tube containing the isolated cells and resuspend in desired medium. Be sure to collect cells from the sides of the tube.	Isolated cells are ready for use

* The appropriate cell concentration will vary depending on the application.