EasySep™ Human FITC Positive Selection Kit

Positive Selection

Catalog #18552

For processing 1 x 10^9 cells

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Description

Isolate highly purified cells labeled with FITC (fluorescein isothiocyanate)-conjugated antibodies from fresh or previously frozen human peripheral blood mononuclear cells (PBMCs) or other single-cell suspensions by immunomagnetic positive selection.

- Fast and easy-to-use
- No columns required

This kit targets cells labeled with FITC-conjugated antibodies (not provided) for positive selection. Desired cells are labeled with antibodies and magnetic particles, and separated without columns using an EasySep™ magnet. Unwanted cells are simply poured off, while desired cells remain in the tube. Isolated cells are immediately available for downstream applications such as flow cytometry, cell culture, or cell-based assays.

Component Descriptions

COMPONENT NAME	COMPONENT #	QUANTITY	STORAGE	SHELF LIFE	FORMAT
EasySep™ FITC Selection Cocktail	18152	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.
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.
Anti-Human CD32 (Fc gamma RII) Blocker, for positive selection	18520	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.
RoboSep™ Vial	18550	1 vial	Not applicable	Not applicable	Not applicable

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

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

If using previously frozen PBMCs, 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 aggreagted suspensions through a 40 µm Cell Strainer (Catalog #27305) for optimal results.

After preparation, resuspend cells at 1 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 mononuclear cells (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 for detailed instructions regarding the EasySep™ procedure for each magnet.

Table 1. EasySep™ Human FITC Positive Selection 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.	1 x 10^8 cells/mL 0.1 - 2.5 mL NOTE: If starting with fewer than 1 x 10^7 cells, resuspend cells in 0.1 mL. For samples with a starting frequency of desired cells < 2%, start with a concentration of 2 x 10^8 cells/mL.	1 x 10^8 cells/mL 0.25 - 8.5 mL NOTE: If starting with fewer than 2.5 x 10^7 cells, resuspend cells in 0.25 mL. For samples with a starting frequency of desired cells < 2%, start with a concentration of 2 x 10^8 cells/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 FcR blocker to sample and mix.	100 µL/mL of sample	100 µL/mL of sample
3	Add FITC-conjugated antibody to sample.‡	0.3 - 3.0 µg/mL of sample	0.3 - 3.0 µg/mL of sample
	Mix and incubate.	RT for 15 minutes	RT for 15 minutes
OPTIONAL WASH STEP may improve performance. Add recommended medium to top up the sample to the indicated volume and centrifuge. Resuspend sample in original volume.		Top up with 10-fold excess recommended medium and centrifuge. Carefully aspirate and discard supernatant. Resuspend in the same volume as step 1.	Top up with 10-fold excess recommended medium and centrifuge. Carefully aspirate and discard supernatant. Resuspend in the same volume as step 1.
4	Add Selection Cocktail to sample.	100 µL/mL of sample	100 µL/mL of sample
	Mix and incubate.	RT for 15 minutes	RT for 15 minutes
5	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
6	Add Magnetic Particles to sample.	50 µL/mL of sample	50 µL/mL of sample
	Mix and incubate.	RT for 10 minutes	RT for 10 minutes
7	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 < 1 mL • Top up to 10 mL for samples ≥ 1 mL
	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 off the supernatant. Remove the tube from the magnet; this tube contains the isolated cells.	Discard supernatant	Discard supernatant
9	Repeat steps as indicated.	Steps 7 and 8, two more times (total of 3 x 5-minute separations)	Steps 7 and 8, two more times (total of 3 x 5-minute separations)
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STEP	INSTRUCTIONS (CONTINUED)	EASYSEP™ MAGNETS  EasySep™ (Catalog #18000)	 "The Big Easy" (Catalog #18001)
OPTIONAL ADDITIONAL SEPARATION(S) **For samples with a starting frequency of desired cells < 15%** **NOTE: This will improve purity but may reduce recovery**		Repeat steps 7 and 8, up to three more times (total of 4 - 6 x 5-minute separations)	Repeat steps 7 and 8, up to three more times (total of 4 - 6 x 5-minute separations)
10	**Resuspend cells in desired medium. Be sure to collect cells from the sides of the tube.**	Isolated cells are ready for use	Isolated cells are ready for use

RT - room temperature (15 - 25°C)
‡ Titrate FITC-conjugated antibody for optimal purity and recovery.
* Recovery may be improved by increasing separation time in the magnet to 10 minutes for each round.
** 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 FITC Positive Selection Kit Protocol

STEP	INSTRUCTIONS	RoboSep™ (Catalog #20000 and #21000) 
1	Prepare sample at the indicated cell concentration within the volume range.	1 x 10^8 cells/Ml 0.25 - 8 Ml NOTE: If starting with fewer than 2.5 x 10^7 cells, resuspend cells in 0.25 mL. For samples with a starting frequency of desired cells < 2%, start with a concentration of 2 x 10^8 cells/mL.
	Add sample to required tube.	14 Ml (17 x 100 mm) polystyrene round-bottom tube (e.g. Corning Catalog #352057)
2	Add FcR blocker to sample and mix.	100 µL/Ml of sample
3	Select protocol.	Human FITC Positive Selection 18552-high purity
4	Transfer FITC-conjugated antibody to the RoboSep™ Vial provided.	Use of this vial is required for RoboSep™ to run properly
5	Mix Magnetic Particles. NOTE: Particles should appear evenly dispersed.	Pipette up and down more than 5 times
6	Load the carousel.	Follow on-screen prompts
	Start the protocol.	Press the green “Run” button
7	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

Notes and Tips

OPTIMIZING PURITY

Purity can be increased, for some cell types, by decreasing the amount of EasySep™ FITC Selection Cocktail added. This may decrease recovery but will also reduce side scatter during subsequent flow cytometry analysis.

OPTIMIZING RECOVERY

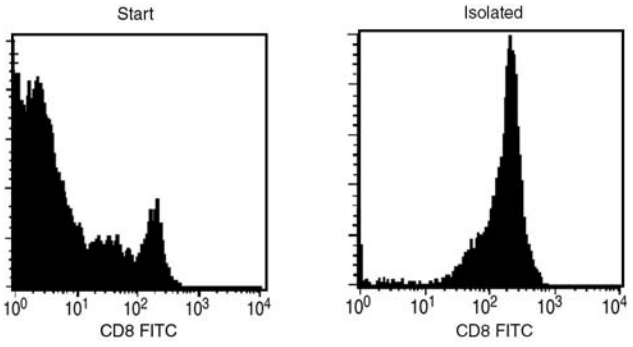
Recovery of positively selected FITC-labeled cells is dependent on the quality of the FITC-conjugated antibody used. Antibodies that have expired or that have been stored improperly may show lower affinity for the surface marker on the target cell, resulting in lower recovery.

It is important to add enough FITC-conjugated antibody to ensure a significant fluorescence intensity of the target cells, as there is a strong correlation between fluorescence intensity and cell recovery. We recommend that the fluorescence intensity of the positively selected cells be at least 100-fold (2 logarithms) greater than that of the negative control for adequate recovery.

ASSESSING PURITY

The positively selected cells have already been FITC-labeled, so the purity can be assessed directly by flow cytometry.

Data



Starting with human PBMCs, the purities of the start and final isolated fractions in the above example are 17.4% and 96.8%, respectively, using a FITC-conjugated anti-human CD8 antibody and EasySep™ Human FITC Positive Selection Kit.