EasySep™ "Do-It-Yourself" Positive Selection Kit II

Positive Selection

Catalog #17698

For processing 1 x 10^9 cells

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Document #DX22368 | Version 1_0_0

Description

Isolate highly purified cell types of interest with your own mouse IgG1 monoclonal antibody from single-cell suspensions by immunomagnetic positive selection.

- Fast and easy-to-use
- No columns

This kit targets any cell type of interest with your own mouse IgG1 monoclonal antibody. 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, culture, or DNA/RNA extraction.

Component Descriptions

COMPONENT NAME	COMPONENT #	QUANTITY	STORAGE	SHELF LIFE	FORMAT
EasySep™ "Do-It-Yourself" Component A	18090	1 x 0.1 mL	Store at 2 - 8°C. Do not freeze.	Stable until expiry date (EXP) on label.	A combination of monoclonal antibodies in PBS.
EasySep™ "Do-It-Yourself" Component B	18091	1 x 0.1 mL	Store at 2 - 8°C. Do not freeze.	Stable until expiry date (EXP) on label.	A combination of monoclonal antibodies in PBS.
EasySep™ Dextran RapidSpheres™ 50100	50100	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.
RoboSep™ Vial for "Do-It-Yourself" Antibody Cocktail	18093	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.

Additional Reagent Stability Information

REAGENT NAME	STORAGE	SHELF LIFE
Selection Cocktail (your own mouse IgG1 monoclonal antibody + Component A + Component B)	Store at 2 - 8°C. Do not freeze.	Stability should be experimentally determined. Do not exceed the shelf life of the individual components.

Sample Preparation

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

PERIPHERAL BLOOD

Prepare a peripheral blood mononuclear cell (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 aggregated 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).

OTHER SAMPLE SOURCES

If other sources or tissues are used, ensure that the cells are in a single-cell suspension. This kit is not recommended for selection of mouse cells.

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 Tables 1 and 2 for detailed instructions regarding the EasySep™ procedure for each magnet.

Table 1. EasySep™ "Do-It-Yourself" Positive Selection Kit II Protocol

		EASYSEP™ MAGNETS	
STEP	INSTRUCTIONS	 **EasySep™ (Catalog #18000)**	**"The Big Easy" (Catalog #18001)** 
1	Add your own mouse IgG1 monoclonal antibody (reconstituted if necessary) to a 1.5 mL polypropylene tube.	15 µg NOTE: Up to 800 µL	15 µg NOTE: Up to 800 µL
2	Add Component A to the polypropylene tube and mix well.	100 µL	100 µL
3	Add Component B to the polypropylene tube.	100 µL	100 µL
	Mix and incubate.	37°C for 5 hours or overnight	37°C for 5 hours or overnight
4	Add sterile PBS to top up the polypropylene tube to the indicated volume.	Top up to 1 mL	Top up to 1 mL
Prepared Selection Cocktail is ready to use.		---	---
5	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 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. Catalog #38007)	14 mL (17 x 95 mm) polystyrene round-bottom tube (e.g. Catalog #38008)
6	Add species-specific FcR blocker (not provided) to sample.	0.5 - 3 µg/mL of sample	0.5 - 3 µg/mL of sample
7	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
8	Vortex RapidSpheres™. NOTE: Particles should appear evenly dispersed.	30 seconds	30 seconds
9	Add RapidSpheres™ to sample.	50 µL/mL of sample	50 µL/mL of sample
	Mix and incubate.	RT for 10 minutes	RT for 10 minutes
Continue on to next page.		Continue on to next page.	Continue on to next page.

		EASYSEP™ MAGNETS	
STEP	INSTRUCTIONS (CONTINUED)	 **EasySep™ (Catalog #18000)**	**"The Big Easy" (Catalog #18001)** 
10	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*
11	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
12	Repeat steps as indicated.	Steps 10 and 11, two more times (total of 3 x 5-minute separations)	Steps 10 and 11, two more times (total of 3 x 5-minute separations)
OPTIONAL ADDITIONAL SEPARATION(S) For samples with a starting frequency of desired cells < 10% NOTE: This will improve purity but may reduce recovery.		Repeat steps 10 and 11, up to three more times (total of 4 - 6 x 5-minute separations)	Repeat steps 10 and 11, up to three more times (total of 4 - 6 x 5-minute separations)
13	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)

* 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.

Table 2. EasySep™ "Do-It-Yourself" Positive Selection Kit II Protocol

		EASYSEP™ MAGNETS	
STEP	INSTRUCTIONS	EasyEights™ (Catalog #18103)	
		5 mL tube	14 mL tube
1	Add your own mouse IgG1 monoclonal antibody (reconstituted if necessary) to a 1.5 mL polypropylene tube.	15 µg NOTE: Up to 800 µL	15 µg NOTE: Up to 800 µL
2	Add Component A to the polypropylene tube and mix well.	100 µL	100 µL
3	Add Component B to the polypropylene tube.	100 µL	100 µL
	Mix and incubate.	37°C for 5 hours or overnight	37°C for 5 hours or overnight
4	Add sterile PBS to top up the polypropylene tube to the indicated volume.	Top up to 1 mL	Top up to 1 mL
Prepared Selection Cocktail is ready to use.		---	---
5	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 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.
	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)
6	Add species-specific FcR blocker (not provided) to sample.	0.5 - 3 µg/mL of sample	0.5 - 3 µg/mL of sample
7	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
8	Vortex RapidSpheres™. NOTE: Particles should appear evenly dispersed.	30 seconds	30 seconds
9	Add RapidSpheres™ to sample.	75 µL/mL of sample	75 µL/mL of sample
	Mix and incubate.	RT for 10 minutes	RT for 10 minutes
Continue on the next page.		Continue on the next page.	Continue on the next page.

		EASYSEP™ MAGNETS	
		EasyEights™ (Catalog #18103)	
STEP	INSTRUCTIONS (CONTINUED)	5 mL tube	14 mL tube
10	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 10 minutes	RT for 10 minutes
11	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
12	Repeat steps as indicated.	Steps 10 and 11, two more times (total of 3 x 10-minute separations)	Steps 10 and 11, two more times (total of 3 x 10-minute separations)
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 10-minute separations)	Repeat steps 7 and 8, up to three more times (total of 4 - 6 x 10-minute separations)
13	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)

** 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 3 for detailed instructions regarding the RoboSep™ procedure.

Table 3. EasySep™ "Do-It-Yourself" Positive Selection Kit II Protocol

STEP	INSTRUCTIONS	RoboSep™ (Catalog #20000 and #21000) 
1	Add your own mouse IgG1 monoclonal antibody (reconstituted if necessary) to the RoboSep™ Vial for "Do-It-Yourself" Antibody Cocktail provided.	15 µg NOTE: Up to 800 µL
2	Add Component A to the RoboSep™ Vial and mix well.	100 µL
3	Add Component B to the RoboSep™ Vial.	100 µL
	Mix and incubate.	37°C for 5 hours or overnight
4	Add sterile PBS to top up the RoboSep™ Vial to the indicated volume.	Top up to 1 mL
Prepared Selection Cocktail is ready to use.		---
5	Prepare sample at the indicated cell concentration within the volume range.	1 x 10^8 cells/mL 0.25 - 8.5 mL NOTE: For samples with a starting frequency of desired cells < 10%, start with a concentration of 2 x 10^8 cells/mL.
	Add sample to required tube.	14 mL (17 x 95 mm) polystyrene round-bottom tube (e.g. Catalog #38008)
6	Add species-specific FcR blocker (not provided) to sample (if desired).	0.5 - 3 µg/mL of sample
7	Select protocol.	Any Species Do-It-Yourself Positive Selection 17698
8	Vortex RapidSpheres™. NOTE: Particles should appear evenly dispersed.	30 seconds
9	Load the carousel.	Follow on-screen prompts
	Start the protocol.	Press the green "Run" button
10	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

ASSESSING PURITY

The mouse IgG1 antibody used in the cocktail created with EasySep™ "Do-It-Yourself" Positive Selection Kit II may block other fluorochrome-conjugated antibodies that are used to assess purity by flow cytometry. Use fluorochrome-conjugated antibody clones that are not blocked by the antibody clone used in the Selection Cocktail. If this is not possible, one of the following methods can be used to assess purity:

- Use alternative fluorochrome-conjugated markers, if applicable.
- Use a fluorochrome-conjugated secondary antibody, such as Goat Anti-Mouse IgG (H+L) Antibody, Polyclonal (Catalog #60138).

ANTIBODY QUALITY

Recovery of positively selected cells is dependent on the quality of the mouse IgG1 antibody used in the cocktail created with EasySep™ "Do-It-Yourself" Positive Selection Kit II. 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.