EasySep™ Anti-Rat IgG2b Positive Selection Kit

Positive Selection

Catalog #18992

For processing 2 x 10^9 cells

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Description

EasySep™ Anti-Rat IgG2b Positive Selection Kit contains all of the necessary components to create a customized EasySep™ selection cocktail when mixed with your own rat IgG2b monoclonal antibody. This selection cocktail can then be used to positively select cells according to the cell surface antigen recognized by your rat IgG2b monoclonal antibody. In addition to positive selection applications, selection components from different EasySep™ Anti-Rat Positive Selection kits can be mixed and matched to make custom enrichment cocktails.

- Fast and easy-to-use
- Up to 99% purity
- No columns required
- Isolated cells are not fluorochrome-labeled

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 or cell culture.

Component Descriptions

COMPONENT NAME	COMPONENT #	QUANTITY	STORAGE	SHELF LIFE	FORMAT
EasySep™ Anti-Rat IgG2b Positive Selection Component	18992C	1 x 0.5 mL	Store at 2 - 8°C. Do not freeze.	Stable until expiry date (EXP) on label.	A combination of monoclonal antibodies in PBS with 5% HPCD.
EasySep™ Dextran RapidSpheres™ 50100	50100	2 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™ Empty Vial	27401	1	Not applicable	Not applicable	Not applicable

HPCD - 2-hydroxypropyl-β-cyclodextrin; 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

Flush bone marrow cells from femur and tibia into recommended medium using a syringe equipped with a 23 gauge needle. Disperse aggregates by gently passing the cell suspension through the syringe several times. Alternatively, crush bones using a mortar and pestle. Remove remaining clumps and debris by passing cell suspension through a 70 µm mesh nylon strainer. Centrifuge at 300 x g for 10 minutes and resuspend cells at 1 x 10^8 cells/mL in recommended medium.

SPLEEN

Disrupt spleen in recommended medium. Remove aggregates and debris by passing cell suspension through a 70 µm mesh nylon strainer. Centrifuge at 300 x g for 10 minutes and resuspend at 1 x 10^8 nucleated cells/mL in recommended medium.

Ammonium chloride treatment is not recommended when preparing the cells for separation.

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™ Anti-Rat IgG2b 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 0.25 - 2 mL	1 x 10^8 cells/mL 0.25 - 8 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	**Dilute your rat IgG2b monoclonal antibody in your buffer of choice in a 1.5 mL polypropylene (microcentrifuge) tube.**	20 µg/mL in buffer	20 µg/mL in buffer
3	**Prepare Selection Cocktail in a tube. For each 1 mL of sample, prepare 50 µL of Selection Cocktail (25 µL of Positive Selection Component + 25 µL of rat IgG2b monoclonal antibody).**	Mix equal volumes of Positive Selection Component and diluted rat IgG2b monoclonal antibody. NOTE: Prepare Selection Cocktail immediately before use.	Mix equal volumes of Positive Selection Component and diluted rat IgG2b monoclonal antibody. NOTE: Prepare Selection Cocktail immediately before use.
	Incubate.	RT for 5 minutes	RT for 5 minutes
4	**Add prepared Selection Cocktail to sample.**	50 µL/mL of sample	50 µL/mL of sample
	Mix and incubate.	RT for 5 minutes	RT for 5 minutes
5	**Vortex RapidSpheres™.** **NOTE: Particles should appear evenly dispersed.**	30 seconds	30 seconds
6	**Add RapidSpheres™ to sample.**	30 µL/mL of sample	50 µL/mL of sample
	Mix and incubate.	RT for 3 minutes	RT for 3 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 < 2 mL • Top up to 10 mL for samples ≥ 2 mL
	Place the tube (without lid) into the magnet and incubate.	RT for 3 minutes	RT for 3 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 3-minute separations)	Steps 7 and 8, two more times (total of 3 x 3-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)

* 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™ Anti-Rat IgG2b Positive Selection Kit Protocol

		EASYSEP™ MAGNETS	
		EasyEights™ (Catalog #18103)	
STEP	INSTRUCTIONS	5 mL tube	14 mL tube
1	Prepare sample at the indicated cell concentration within the volume range.	1 x 10^8 cells/mL 0.25 - 1 mL	1 x 10^8 cells/mL 0.5 - 8 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	Dilute your rat IgG2b monoclonal antibody in your buffer of choice in a 1.5 mL polypropylene (microcentrifuge) tube.	20 µg/mL in buffer	20 µg/mL in buffer
3	Prepare Selection Cocktail in a tube. For each 1 mL of sample, prepare 50 µL of Selection Cocktail (25 µL of Positive Selection Component + 25 µL of rat IgG2b monoclonal antibody).	Mix equal volumes of Positive Selection Component and diluted rat IgG2b monoclonal antibody. NOTE: Prepare Selection Cocktail immediately before use.	Mix equal volumes of Positive Selection Component and diluted rat IgG2b monoclonal antibody. NOTE: Prepare Selection Cocktail immediately before use.
	Incubate.	RT for 5 minutes	RT for 5 minutes
4	Add prepared Selection Cocktail to sample.	50 µL/mL of sample	50 µL/mL of sample
	Mix and incubate.	RT for 5 minutes	RT for 5 minutes
5	Vortex RapidSpheres™. NOTE: Particles should appear evenly dispersed.	30 seconds	30 seconds
6	Add RapidSpheres™ to sample.	50 µL/mL of sample	50 µL/mL of sample
	Mix and incubate.	RT for 3 minutes	RT for 3 minutes
7	Add recommended medium to top up sample to the indicated volume. Mix by gently pipetting up and down 2 - 3 times.	Top up to 2.5 mL	• Top up to 3 mL for samples < 2 mL • Top up to 10 mL for samples ≥ 2 mL
	Place the tube (without lid) into the magnet and incubate.	RT for 10 minutes	RT for 10 minutes
8	Carefully pipette** (do not pour) off the supernatant. Remove the tube from the magnet; this tube contains the isolated cells.	Discard supernatant	Discard supernatant
9	Add recommended medium to top up sample to the indicated volume. Mix by gently pipetting up and down 2 - 3 times.	Top up to 2.5 mL	• Top up to 3 mL for samples < 2 mL • Top up to 10 mL for samples ≥ 2 mL
	Place the tube (without lid) into the magnet and incubate.	RT for 5 minutes	RT for 5 minutes
10	Carefully pipette** (do not pour) off the supernatant. Remove the tube from the magnet; this tube contains the isolated cells.	Discard supernatant	Discard supernatant
11	Repeat steps as indicated.	Steps 9 and 10 (total of 1 x 10-minute and 2 x 5-minute separations)	Steps 9 and 10 (total of 1 x 10-minute and 2 x 5-minute separations)
12	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. RoboSep™ Anti-Rat IgG2b 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 0.5 - 8 mL
	Add sample to required tube.	14 mL (17 x 95 mm) polystyrene round-bottom tube (e.g. Catalog #38008)
2	Dilute your rat IgG2b monoclonal antibody in your buffer of choice in a 1.5 mL polypropylene (microcentrifuge) tube.	20 µg/mL in buffer
3	Prepare Selection Cocktail in the RoboSep™ Empty Vial provided. See Table 4 for required volumes.	Mix equal volumes of Positive Selection Component and diluted rat IgG2b monoclonal antibody. NOTE: Prepare Selection Cocktail immediately before use.
	Incubate.	RT for 5 minutes
4	Select protocol.	Anti-Rat IgG2b Positive Selection 18992
5	Vortex RapidSpheres™. NOTE: Particles should appear evenly dispersed.	30 seconds
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

Table 4. RoboSep™ Selection Cocktail Preparation

START SAMPLE	POSITIVE SELECTION COMPONENT	RAT IgG2b MONOCLONAL ANTIBODY	PREPARED SELECTION COCKTAIL TOTAL VOLUME
0.5 mL	62.5 µL	62.5 µL	125 µL
1 mL	75 µL	75 µL	150 µL
1.5 mL	87.5 µL	87.5 µL	175 µL
2 mL	100 µL	100 µL	200 µL
3 mL	125 µL	125 µL	250 µL
4 mL	150 µL	150 µL	300 µL
5 mL	175 µL	175 µL	350 µL
6 mL	200 µL	200 µL	400 µL
7 mL	225 µL	225 µL	450 µL
8 mL	250 µL	250 µL	500 µL

Note: RoboSep™ requires an extra 100 µL of the Selection Cocktail to run properly (compared to manual protocols).

Notes and Tips

ASSESSING PURITY

The rat IgG2b antibody used in the cocktail created with EasySep™ Anti-Rat IgG2b Positive Selection Kit 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 rat antibody used in the selection cocktail. If this is not possible, one of the following methods can be used to assess purity:

- Add a fluorochrome-conjugated antibody at a concentration of 0.5 µg/mL immediately after adding the cocktail. This method labels the positive cells in the entire sample.
- 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).

OPTIMIZING RECOVERY

Recovery may be improved by increasing separation time in the magnet from 3 to 10 minutes for each round. Recovery of positively selected cells is also dependent on the quality of the rat IgG2b antibody used in the cocktail created with EasySep™ Anti-Rat IgG2b Positive Selection Kit. 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.

OPTIMIZING PURITY

For samples with a desired cell starting frequency of less than 10 - 15%, additional separation rounds will likely improve purity. If desired, repeat Steps 7 and 8 (Tables 1 and 2) an additional 1 to 3 times. Note that recovery will decrease with each additional round of separation. Also, for rare cells (i.e., cells representing less than 5% of the initial population), increasing initial cell concentration from 1 x 10^8 cells/mL to 2 x 10^8 cells/mL may improve purity.

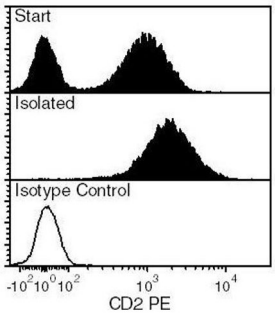
OPTIMIZING PERFORMANCE

In some cases, titration of the rat IgG2b antibody and EasySep™ Dextran RapidSpheres™ may be required for optimal performance. The concentration of the rat IgG2b antibody may be increased or decreased from the recommended 20 µg/mL to achieve optimal purity and recovery, and EasySep™ Dextran RapidSpheres™ may also be increased from 40 µL/mL, as needed. The volume of Positive Selection Component incubated with the chosen rat IgG2b antibody can also be increased or decreased from the recommended 1:1 ratio.

PREVENTING NONSPECIFIC BINDING

To prevent nonspecific binding of antibodies to cellular FcR receptors, Normal Rat Serum may be added to samples at 50 µL/mL of cells.

Data



Flow cytometry analysis of mouse splenocytes processed with EasySep™ Anti-Rat IgG2b Positive Selection Kit using a PE-conjugated rat IgG2b anti-mouse CD2 antibody as the primary antibody for cell marker selection and simultaneous labeling (see Notes and Tips). Histograms show labeling of splenocytes (Start) and isolated cells (Isolated). Labeling of start cells with Rat IgG2b, kappa Isotype Control Antibody, Clone RTK4530, PE (Catalog #60077PE) is shown in the bottom panel (solid line histogram).