EasySep™ Negative Selection

Catalog #19860

EasySep™ Mouse Streptavidin RapidSpheres™ Isolation Kit

For processing 1 x 10^9 cells

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Description

Isolate untouched cells through depletion of select cell types from mouse splenocytes or other single-cell suspensions by immunomagnetic negative selection.

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

This kit targets unwanted cells for removal with biotinylated antibodies (not provided) recognizing specific cell surface markers. Unwanted cells are labeled with biotinylated antibodies and streptavidin-coated 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.

This kit is not recommended for positive selection of mouse cells. For positive selection, use the EasySep™ Mouse Biotin Selection Kit (Catalog #18556).

Component Descriptions

COMPONENT NAME	COMPONENT #	QUANTITY	STORAGE	SHELF LIFE	FORMAT
EasySep™ Streptavidin RapidSpheres™ 50001	50001	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 PBS.
Normal Rat Serum	13551	1 x 2 mL	Store at -20°C.	Stable until expiry date (EXP) on label.	Mycoplasma-free normal rat serum.
RoboSep™ Empty Vial	27401	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
Normal Rat Serum (in-use)	Store at 2 - 8°C.	Stable for at least 2 months. Do not exceed expiry date (EXP) on label.

Sample Preparation

SPLEEN

Disrupt spleen in PBS containing 2% fetal bovine serum (FBS). Remove clumps 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% FBS and 1 mM EDTA. Medium should be free of Ca++, Mg++, and biotin.

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™ Mouse Streptavidin RapidSpheres™ Isolation 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 mL	1 x 10^8 cells/mL 0.5 - 8 mL
2	**Add Rat Serum to sample.**	50 µL/mL of sample	50 µL/mL of sample
3	**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)
OPTIONAL: If there is non-specific binding of antibodies to FcR receptors, add a species-specific FcR blocking antibody or IgG.		0.3 - 3 µg/mL of sample	0.3 - 3 µg/mL of sample
4	**Add each biotinylated antibody to sample.** **NOTE: The biotinylated antibodies should be titrated.**	0.5 - 5 µg/mL of sample when using multiple biotinylated antibodies 5 µg/mL of sample when using a single biotinylated antibody	0.5 - 5 µg/mL of sample when using multiple biotinylated antibodies 5 µg/mL of sample when using a single biotinylated antibody
	Mix and incubate.	RT for 10 minutes	RT for 10 minutes
5	**Vortex RapidSpheres™.**	30 seconds	30 seconds
6	**Add RapidSpheres™ to sample.** **NOTE: The RapidSpheres™ should be titrated.**	25 - 75 µL/mL of sample for low frequency (< 30%) unwanted cells 75 - 125 µL/mL of sample for high frequency (> 70%) unwanted cells	25 - 75 µL/mL of sample for low frequency (< 30%) unwanted cells 75 - 125 µL/mL of sample for high frequency (> 70%) unwanted cells
	Mix and incubate.	RT for 2.5 minutes	RT for 2.5 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 < 4 mL • Top up to 10 mL for samples ≥ 4 mL
	Place the tube (without lid) into the magnet and incubate.	RT for 2.5 minutes NOTE: May be increased to 5 minutes. This will improve purity but may reduce recovery.	RT for 2.5 minutes NOTE: May be increased to 5 minutes. This will improve purity but may reduce recovery.
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 new 5 mL tube Isolated cells are ready for use	Use new 14 mL tube Isolated cells are ready for use
OPTIONAL ADDITIONAL SEPARATION for PURITY **NOTE: This will improve purity but may reduce recovery.**		---	---
9	**Remove the tube from the magnet and place the new tube (without lid) into the magnet and incubate for a second separation.**	RT for 2.5 minutes	RT for 2.5 minutes
10	**Pick up the magnet, and in one continuous motion invert the magnet and tube,* pouring the enriched cell suspension.**	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.

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™ Mouse Streptavidin RapidSpheres™ Isolation 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
2	Add Rat Serum to sample.	50 µL/mL of sample
3	Add sample to required tube.	14 mL (17 x 100 mm) polystyrene round-bottom tube (e.g. Corning Catalog #352057)
4	Prepare a cocktail of biotinylated antibodies at a 20-fold greater concentration than desired (typically 0.5 - 5 µg/mL of sample for each biotinylated antibody) in the RoboSep™ Empty Vial. NOTE: The biotinylated antibodies should be titrated.	Minimum cocktail volume will be indicated on the RoboSep™ screen
OPTIONAL: If there is non-specific binding of antibodies to FcR receptors, add a species-specific FcR blocking antibody or IgG.		0.3 - 3 µg/mL of sample
5	Select protocol.	Mouse Streptavidin RapidSpheres Isolation 19860
6	Vortex RapidSpheres™.	30 seconds
7	Load the carousel.	Follow on-screen prompts
	Start the protocol.	Press the green “Run” button
8	Unload the carousel when the run is complete. Remove the tube containing the isolated cells.	Isolated cells are ready for use

Notes and Tips

BIOTINYLATED ANTIBODY SELECTION

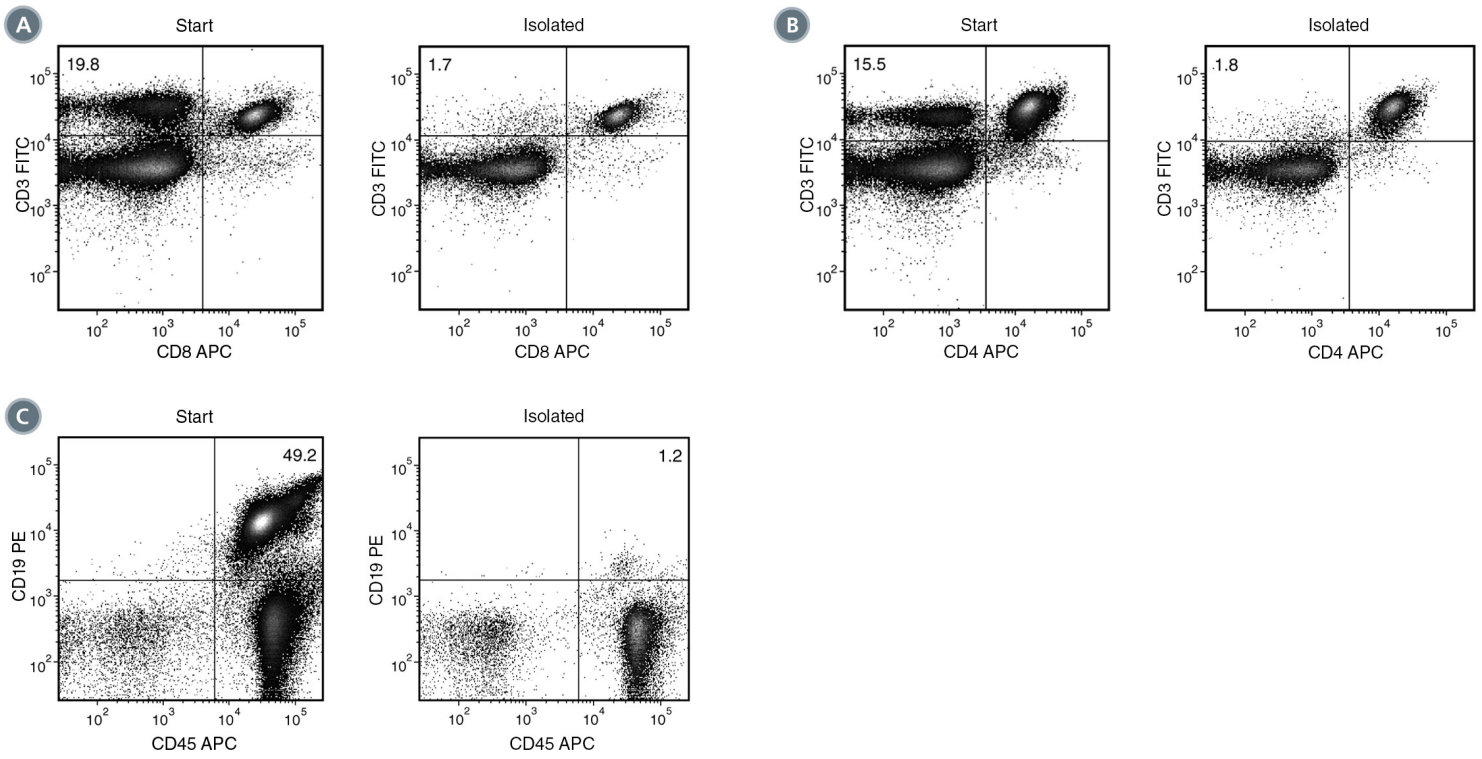
The choice of biotinylated antibodies used is very important for cell separation performance. Their quality should be assessed using flow cytometry and labeling with fluorochrome-conjugated streptavidin or fluorochrome-conjugated anti-biotin antibodies (not provided).

ASSESSING PURITY

For purity assessment of unwanted cells by flow cytometry use fluorochrome-conjugated antibodies. If the biotinylated antibodies block the labeling antibody, use an alternative marker.

For a complete list of antibodies, please visit our website at www.stemcell.com/antibodies or contact STEMCELL Technologies Technical Support at techsupport@stemcell.com.

Data



(A) Typical Mouse Streptavidin Rapidspheres™ CD4 (CD3+CD8-) depletion profile.

(B) Typical Mouse Streptavidin Rapidspheres™ CD8 (CD3+CD4-) depletion profile.

(C) Typical Mouse Streptavidin Rapidspheres™ CD19 (CD19+CD45+) depletion profile.