

# EasySep™ Mouse Streptavidin RapidSpheres™ Isolation Kit



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For processing  $1 \times 10^9$  cells

Catalog #19860  
 #19860RF RoboSep™

Negative Selection

Document #10000003757 | Version 02

## 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 EasySep™ Mouse Biotin Positive Selection Kit II (Catalog #17665).

## 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.
EasySep™ Mouse FcR Blocker	18731	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, 0.1% BSA, and < 0.1% sodium azide.
RoboSep™ Empty Vial	27401	1 vial	Not applicable	Not applicable	Not applicable

BSA - bovine serum albumin; PBS - phosphate-buffered saline

Components may be shipped at room temperature (15 - 25°C) but should be stored as indicated above.

## Sample Preparation

For automated and standardized tissue processing, see STEMprep™ Tissue Dissociator (Catalog #100-2112) at [www.stemcell.com/stemprep](http://www.stemcell.com/stemprep). For manual processing, follow the steps below.

### SPLEEN

Disrupt spleen in PBS containing 2% fetal bovine serum (FBS). Remove aggregates and debris by passing cell suspension through a 70 µm mesh nylon strainer (e.g. Catalog #27216). Centrifuge at 300 x g for 10 minutes and resuspend at  $1 \times 10^8$  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<sup>++</sup>, Mg<sup>++</sup>, 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 <sup>8</sup> cells/mL 0.1 - 2 mL	1 x 10 <sup>8</sup> cells/mL 0.5 - 8 mL
2	Add FcR blocker to sample. NOTE: Titration of FcR blocker may be required.	20 µL/mL of sample	20 µL/mL of sample
3	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)
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™. NOTE: Particles should appear evenly dispersed.	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	<ul style="list-style-type: none"> <li>• Top up to 5 mL for samples &lt; 4 mL</li> <li>• Top up to 10 mL for samples ≥ 4 mL</li> </ul>
	Place the tube (without lid) into the magnet and incubate.	RT for 2.5 minutes*	RT for 2.5 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.	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 into a new tube.	Isolated cells are ready for use	Isolated cells are ready for use

RT - room temperature (15 - 25°C)

\* Purity may be improved by increasing separation time in the magnet to 5 minutes. NOTE: This will improve purity but may reduce recovery.

\*\* 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 #21000) 
1	Prepare sample at the indicated cell concentration within the volume range.	1 x 10 <sup>8</sup> cells/mL 0.5 - 8 mL
2	Add FcR blocker to sample. NOTE: Titration of FcR blocker may be required.	20 µL/mL of sample
3	Add sample to required tube.	14 mL (17 x 95 mm) polystyrene round-bottom tube (e.g. Catalog #38008)
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™. NOTE: Particles should appear evenly dispersed.	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.	Isolated cells are ready for use

## Notes and Tips

### BIOTINYLATED ANTIBODY SELECTION

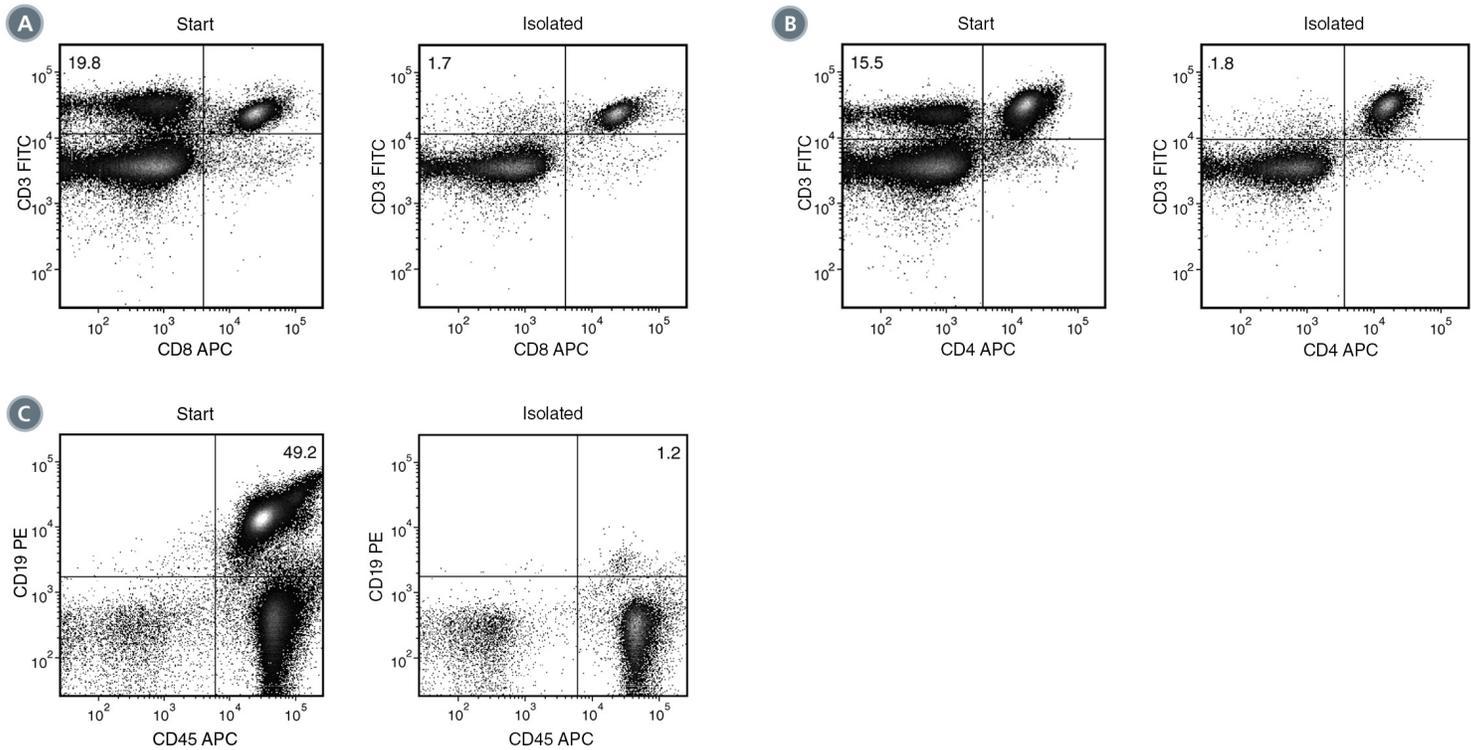
The choice of biotinylated antibodies used is important for cell separation performance. Their quality should be assessed using flow cytometry and labeling with fluorochrome-conjugated streptavidin or fluorochrome-conjugated anti-biotin antibodies (not provided).

### 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, visit [www.stemcell.com/antibodies](http://www.stemcell.com/antibodies), or contact us at [techsupport@stemcell.com](mailto:techsupport@stemcell.com).

## Data



**Figure 1. Typical Mouse Streptavidin RapidSpheres™ (A) CD4 (CD3+CD8-), (B) CD8 (CD3+CD4-), or (C) CD19 (CD19+CD45+) Depletion Profiles**

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