

# EasySep™ Mouse NK Cell Isolation Kit

For processing 1 x 10<sup>9</sup> cells

Catalog #19855

Catalog #19855RF RoboSep™

Negative Selection

Document #1000000949 | Version 02



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## Description

Isolate untouched and highly purified NK cells from mouse splenocytes by immunomagnetic negative selection. When using single-cell suspensions from other tissue types, this kit may require optimization.

- Fast and easy-to-use
- No columns required
- Untouched, viable cells

This kit targets non-NK cells for removal with biotinylated antibodies 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 cell-based assays.

## Component Descriptions

COMPONENT NAME	COMPONENT #	QUANTITY	STORAGE	SHELF LIFE	FORMAT
EasySep™ Mouse NK Cell Isolation Cocktail	19855C	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 and 0.1% BSA.
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.

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

### SPLEEN

Disrupt spleen in PBS or Hanks' Balanced Salt Solution (HBSS) containing 2% fetal bovine serum (FBS). Remove clumps and debris by passing cell suspension through a 70 µm Reversible Strainer (e.g. Catalog #27216). Centrifuge at 300 x g for 10 minutes and resuspend at 1 x 10<sup>8</sup> 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. HBSS, Modified (without Ca<sup>++</sup> and Mg<sup>++</sup>; Catalog #37250) can be used in place of PBS. 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 Tables 1 and 2 for detailed instructions regarding the EasySep™ procedure for each magnet.

**Table 1. EasySep™ Mouse NK Cell 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 0.5 - 2 mL	1 x 10 <sup>8</sup> cells/mL 1 - 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	Add Isolation Cocktail to sample. NOTE: Do not vortex cocktail.	50 µL/mL of sample	50 µL/mL of sample
	Mix and incubate.	RT for 10 minutes	RT for 10 minutes
3	Vortex RapidSpheres™. NOTE: Particles should appear evenly dispersed.	30 seconds	30 seconds
4	Add RapidSpheres™ to sample.	100 µL/mL of sample	100 µL/mL of sample
	Mix and incubate.	RT for 5 minutes	RT for 5 minutes
5	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 5 minutes	RT for 5 minutes
6	Pick up the magnet, and in one continuous motion invert the magnet and tube,* pouring the enriched cell suspension into a new tube and set aside.	Use a new 14 mL tube	<ul style="list-style-type: none"> <li>• Use a new 14 mL tube for start samples &lt; 4 mL</li> <li>• Use a new 50 mL tube for start samples ≥ 4 mL</li> </ul>
7	Remove the tube from the magnet and add recommended medium to the indicated volume. Mix by gently pipetting up and down 5 - 6 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 5 minutes	RT for 5 minutes
8	Pick up the magnet, and in one continuous motion invert the magnet and tube,* pouring off the enriched cell suspension.	Combine with first poured-off fraction from step 6 Isolated cells are ready for use	Combine with first poured-off fraction from step 6 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.

The protocol for isolating NK cells using the EasyEights™ magnet can be adjusted to achieve higher final purity of the target cells. For more information, contact us at techsupport@stemcell.com.

**Table 2. EasySep™ Mouse NK Cell Isolation Kit Protocol**

		EASYSEP™ MAGNETS	
STEP	INSTRUCTIONS	EasyEights™ (Catalog #18103)	
		5 mL tube	14 mL tube
1	Prepare sample at the indicated cell concentration within the volume range.	1 x 10 <sup>8</sup> cells/mL 0.5 - 2 mL	1 x 10 <sup>8</sup> cells/mL 1 - 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	Add Isolation Cocktail to sample. NOTE: Do not vortex cocktail.	50 µL/mL of sample	50 µL/mL of sample
	Mix and incubate.	RT for 10 minutes	RT for 10 minutes
3	Vortex RapidSpheres™. NOTE: Particles should appear evenly dispersed.	30 seconds	30 seconds
4	Add RapidSpheres™ to sample.	100 µL/mL of sample	100 µL/mL of sample
	Mix and incubate.	RT for 5 minutes	RT for 5 minutes
5	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	<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 5 minutes	RT for 5 minutes
6	Carefully pipette** (do not pour) the enriched cell suspension into a new tube and set aside.	Use a new 14 mL tube	<ul style="list-style-type: none"> <li>• Use a new 14 mL tube for start samples &lt; 4 mL</li> <li>• Use a new 50 mL tube for start samples ≥ 4 mL</li> </ul>
7	Remove the tube from the magnet and add recommended medium to the indicated volume. Mix by gently pipetting up and down 5 - 6 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 5 minutes	RT for 5 minutes
8	Carefully pipette** (do not pour) off the enriched cell suspension.	Combine with first fraction from step 6 Isolated cells are ready for use	Combine with first fraction from step 6 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™ Mouse NK Cell 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 1 - 8 mL NOTE: If starting with fewer than 1 x 10 <sup>8</sup> cells, resuspend cells in 1 mL.	
	Add sample to required tube.	14 mL (17 x 95 mm) polystyrene round-bottom tube (e.g. Catalog #38008)	
2	Select protocol.	Mouse NK Cell Isolation 19855	
3	Vortex RapidSpheres™. NOTE: Particles should appear evenly dispersed.	30 seconds	
4	Load the carousel.	Follow on-screen prompts	
	Start the protocol.	Press the green “Run” button	
5	Unload the carousel when the run is complete.	Isolated cells are ready for use	

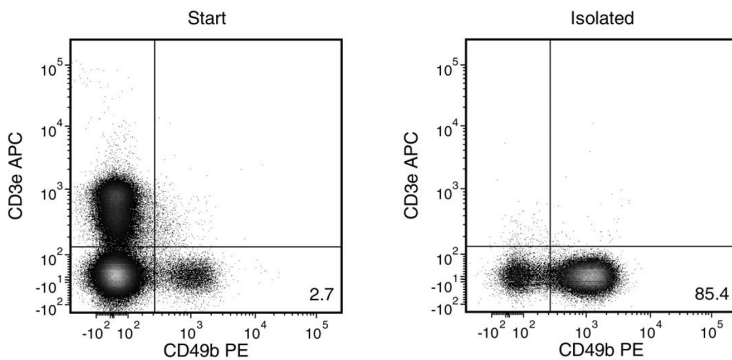
## Notes and Tips

### ASSESSING PURITY

For purity assessment of NK cells by flow cytometry, use the following fluorochrome-conjugated antibody clones:

- Anti-Mouse CD3e Antibody, Clone 145-2C11 (Catalog #60015), and
- Anti-Mouse CD49b Antibody, Clone DX5 (Catalog #60020)

## Data



Starting with mouse splenocytes, the NK cell content (CD3-CD49b+) of the isolated fraction typically ranges from 67 - 89%. In the above example, the purities of the start and final isolated fractions are 2.7% and 85.4%, respectively.

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