EasySep™ Mouse NK Cell Enrichment Kit

Negative Selection

Catalog #19755

For processing 1 x 10^9 cells

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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, easy-to-use and column-free
- Up to 87% purity
- Untouched, viable cells

This kit targets non-NK cells for removal with biotinylated antibodies recognizing non-NK cell surface marker. Unwanted cells are labeled with biotinylated antibodies and 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.

Component Descriptions

COMPONENT NAME	COMPONENT #	QUANTITY	STORAGE	SHELF LIFE	FORMAT
EasySep™ Negative Selection Mouse NK Cell Enrichment Cocktail	19755C.2	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™ Biotin Selection Cocktail	19153	2 x 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™ D Magnetic Particles	19250	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 TBS.

PBS - phosphate-buffered saline; TBS - TRIS-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 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% FBS and 1 mM EDTA. HBSS, Modified (Without Ca++ and Mg++; 37250) can be used in place of PBS. 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 Table 1 for detailed instructions regarding the EasySep™ procedure for each magnet.

Table 1. EasySep™ Mouse NK Cell Enrichment 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 0.5 - 1.5 mL	1 x 10^8 cells/mL 1 - 5 mL
	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)
2	**Add Enrichment Cocktail to sample.**	50 µL/mL of sample	50 µL/mL of sample
	Mix and incubate.	RT for 15 minutes	RT for 15 minutes
3	**Add Selection Cocktail to sample.**	200 µL/mL of sample	200 µL/mL of sample
	Mix and incubate.	RT for 15 minutes	RT for 15 minutes
4	**Vortex Magnetic Particles.** **NOTE: Particles should appear evenly dispersed.**	30 seconds	30 seconds
5	**Add Magnetic Particles to sample.**	200 µL/mL of sample	200 µL/mL of sample
	Mix and incubate.	RT for 10 minutes	RT for 10 minutes
6	**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 5 minutes	RT for 5 minutes
7	**Pick up the magnet, and in one continuous motion invert the magnet and tube,* pouring the enriched cell suspension into a new tube.**	Use a new 5 mL tube	Use a new 14 mL tube
8	**Place the new tube (without lid) into the magnet and incubate.**	RT for 5 minutes	RT for 5 minutes
9	**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). For more information about the optimal temperature of the protocol, see Note and Tips on page 3.
* 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.

Notes and Tips

ASSESSING PURITY

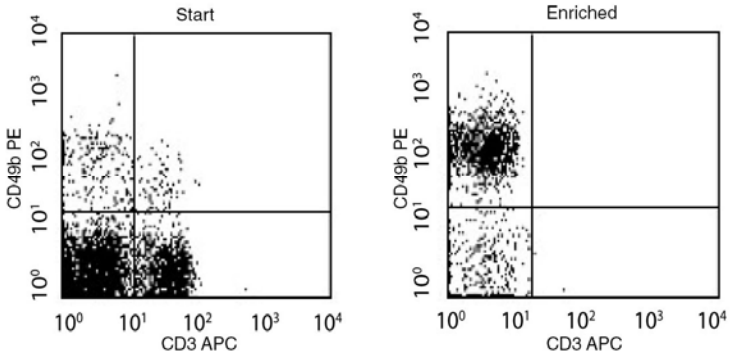
For purity assessment of NK cells (CD49b+CD3-) by flow cytometry use the following fluorochrome-conjugated antibody clones:

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

OPTIMAL TEMPERATURE

This procedure has been optimized at room temperature (15 - 25°C). However, it can also be performed at 2 - 8°C by pre-chilling cell suspension, recommended medium, and magnet, and by performing all incubation steps in a refrigerator. Under these conditions, we strongly recommend that 5% normal rat serum be added to the cells prior to enrichment. The serum is used to prevent non-specific binding of rat antibodies to mouse cells. Please note that no significant improvement to product performance or cell viability was found using these conditions compared to the standard room temperature protocol.

Data



Starting with C57BL/6 mouse splenocytes, the NK cell content (CD49b+CD3-) of the enriched fraction typically ranges from 75 - 87%. In the above example, the purities of the start and final enriched fractions are 3.7% and 79.7%, respectively.