EasySep™ Mouse Neutrophil Enrichment Kit

For processing 1 x 10^9 cells

Catalog #19762

Negative Selection

Document #10000003730 | Version 01

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Description

Isolate untouched and highly purified neutrophils from mouse bone marrow or peripheral blood 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 93.7% (blood) and 88.7% (bone marrow) purity
- Isolated cells are untouched

This kit targets non-neutrophils for removal with biotinylated antibodies recognizing specific cell surface markers. 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, cell culture, or cell-based assays.

Component Descriptions

COMPONENT NAME	COMPONENT #	QUANTITY	STORAGE	SHELF LIFE	FORMAT
EasySep™ Mouse Neutrophil Enrichment Cocktail	19762C	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	1 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.
Normal Rat Serum	13551	1 x 2 mL	Store at -20°C.	Stable until expiry date (EXP) on label.	Mycoplasma-free normal rat serum.

BSA - bovine serum albumin; 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.

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

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 aggregates 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 nucleated cells/mL in recommended medium. Ammonium chloride treatment is not recommended when preparing the cells for separation.

PERIPHERAL BLOOD

Blood should be lysed prior to use. Mix 1 part blood with 9 parts Ammonium Chloride Solution (Catalog #07800) and incubate on ice for 15 minutes. Centrifuge at 300 x g for 6 minutes. Discard supernatant and wash cell pellet once with recommended medium. Discard supernatant and resuspend cell pellet at 1 x 10^8 nucleated cells/mL in recommended medium. If there are less than 5 x 10^7 nucleated cells/mL, resuspend in 500 µL of recommended medium.

Recommended Medium

EasySep™ Buffer (Catalog #20144), RoboSep™ Buffer (Catalog #20104), or PBS containing 2% fetal bovine serum (FBS) and 1 mM EDTA. HBSS, Modified (Without Ca++ and Mg++; Catalog #37250) can be used in place of PBS (Catalog #37350). 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 Neutrophil 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 - 2 mL NOTE: If using < 5 x 10^7 cells, top up to 0.5 mL with recommended medium	1 x 10^8 cells/mL 0.5 - 6.5 mL NOTE: If using < 5 x 10^7 cells, top up to 0.5 mL with recommended medium
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. Catalog #38007)	14 mL (17 x 95 mm) polystyrene round-bottom tube (e.g. Catalog #38008)
4	**Add Enrichment Cocktail to sample.** **NOTE: Do not vortex cocktail.**	50 µL/mL of sample	50 µL/mL of sample
	Mix and incubate.	2 - 8°C for 15 minutes	2 - 8°C for 15 minutes
5	**Wash the cells by topping up the sample tube with recommended medium and centrifuge.**	300 x g for 10 minutes	300 x g for 10 minutes
	Discard the supernatant and resuspend cells in the original volume with recommended medium.	0.5 - 2 mL	0.5 - 6.5 mL
6	**Add Biotin Selection Cocktail to sample.** **NOTE: Do not vortex cocktail.**	50 µL/mL of sample	50 µL/mL of sample
	Mix and incubate.	2 - 8°C for 15 minutes	2 - 8°C for 15 minutes
7	**Vortex Magnetic Particles.** **NOTE: Particles should appear evenly dispersed.**	30 seconds	30 seconds
8	**Add Magnetic Particles to sample.**	150 µL/mL of sample	150 µL/mL of sample
	Mix and incubate.	2 - 8°C for 10 minutes	2 - 8°C for 10 minutes
9	**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 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)

* 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.

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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 Neutrophil Enrichment Kit Protocol

STEP	INSTRUCTIONS	RoboSep™ (Catalog #21000) 
1	Prepare sample at the indicated cell concentration within the volume range.	1 x 10^8 cells/mL 0.5 - 6.5 mL NOTE: If using < 5 x 10^7 cells, top up to 0.5 mL with recommended medium
2	Add Rat Serum to sample.	50 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	Add Enrichment Cocktail to sample. NOTE: Do not vortex cocktail.	50 µL/mL of sample
	Mix and incubate.	2 - 8°C for 15 minutes
5	Wash the cells by topping up the sample tube with recommended medium and centrifuge.	300 x g for 10 minutes
	Discard the supernatant and resuspend cells in the original volume with recommended medium.	0.5 - 6.5 mL
6	Select protocol.	Mouse Neutrophil Negative Selection 19762
7	Vortex Magnetic Particles. NOTE: Particles should appear evenly dispersed.	30 seconds
8	Load the carousel.	Follow on-screen prompts
	Start the protocol.	Press the green “Run” button
9	Unload the carousel when the run is complete. Remove the tube containing the isolated cells.	Isolated cells are ready for use

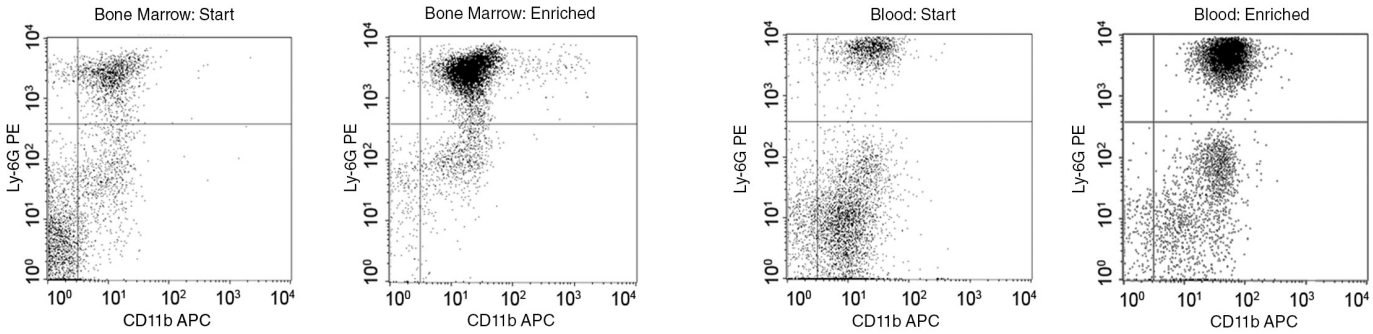
Notes and Tips

ASSESSING PURITY

To date, an exclusive marker for mouse bone marrow and peripheral blood neutrophils has not been identified. However, neutrophils are known to express CD11b, Gr-1, Ly-6C, and Ly-6G. For purity assessment of neutrophils (CD11b+Ly-6G+) by flow cytometry, use the following fluorochrome-conjugated antibodies:

- Anti-Mouse CD11b Antibody, Clone M1/70 (Catalog #60001), and
- Anti-Mouse Ly-6G Antibody, Clone 1A8 (Catalog #60031)

Data



Starting with mouse bone marrow or mouse blood, the CD11b+Ly-6G+ cell content of the enriched fraction typically ranges from 69.9 - 88.7% (bone marrow) or 80.1 - 93.7% (blood). In the above example, the purities of the start and final enriched fractions are 25.2% and 86.9% (bone marrow), and 22.1% and 90.1% (blood), respectively.