

# EasySep™ Mouse Neutrophil Enrichment Kit

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

Catalog #19762  
#19762RF RoboSep™

Negative Selection

Document #10000003730 | Version 02



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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% (blood) and 91% (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.
EasySep™ Mouse FcR Blocker	18730	1 x 0.2 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.

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.

## 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<sup>8</sup> 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<sup>8</sup> nucleated cells/mL in recommended medium. If there are less than 5 x 10<sup>7</sup> 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<sup>++</sup> and Mg<sup>++</sup>; Catalog #37250) can be used in place of PBS (Catalog #37350). Medium should be free of Ca<sup>++</sup> and Mg<sup>++</sup>.

## 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 <sup>8</sup> cells/mL 0.5 - 2 mL NOTE: If using < 5 x 10 <sup>7</sup> cells, top up to 0.5 mL with recommended medium	1 x 10 <sup>8</sup> cells/mL 0.5 - 6.5 mL NOTE: If using < 5 x 10 <sup>7</sup> cells, top up to 0.5 mL with recommended medium
2	Add FcR Blocker to sample.	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)
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	<ul style="list-style-type: none"> <li>• Top up to 5 mL for samples ≤ 2 mL</li> <li>• Top up to 10 mL for samples &gt; 2 mL</li> </ul>
	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.

## 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 <sup>8</sup> cells/mL 0.5 - 6.5 mL NOTE: If using < 5 x 10 <sup>7</sup> cells, top up to 0.5 mL with recommended medium
2	Add FcR Blocker to sample.	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	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

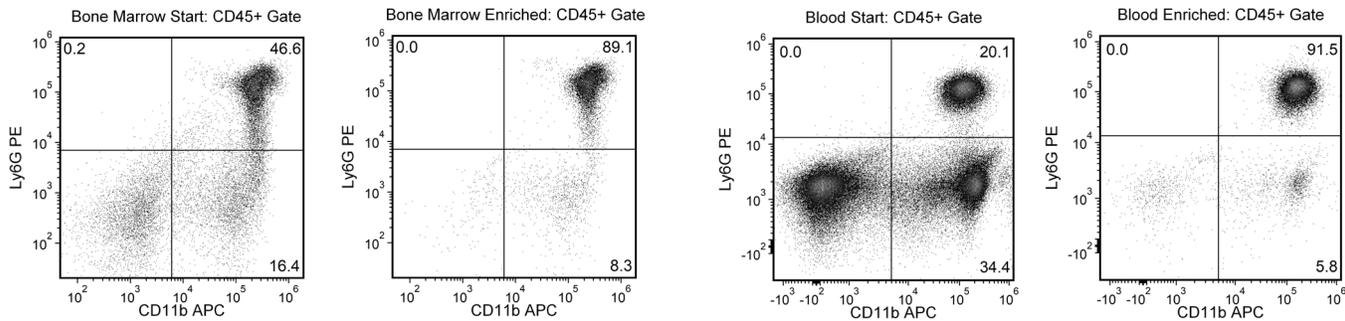
## Notes and Tips

### 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+Ly6G+ cell content of the enriched fraction is typically 88.2 ± 3.2% for bone marrow and 88.6 ± 4.9% for blood (mean ± SD using the purple EasySep™ magnet). In the above example, the purities of the start and final enriched fractions are 46.6% and 89.1% (bone marrow) and 20.1% and 91.5% (blood), respectively.

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