# EasySep™ Mouse Monocyte Enrichment Kit

Negative Selection

Catalog #19761

For processing 1 x 10^9 cells

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## Description

Isolate untouched and highly purified monocytes from mouse bone marrow or peripheral blood samples 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 98% purity (blood), 93% purity (bone marrow)
- Isolated cells are untouched

This kit targets non-monocytes for removal with biotinylated antibodies recognizing specific cells 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, culture, or DNA/RNA extraction.

## Component Descriptions

| COMPONENT NAME | COMPONENT # | QUANTITY | STORAGE | SHELF LIFE | FORMAT |
|---|---|---|---|---|---|
| EasySep™ Mouse Monocyte Enrichment Cocktail | 19761C.1 | 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 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 clumps by gently passing the cell suspension through the syringe several times. Alternatively, crush bones using a mortar and pestle. Remove remaining clumps 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 cells/mL in recommended medium.

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 cells/mL in recommended medium.

If there are less than 5 x 10^7 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. 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 Monocyte 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<br>0.5 - 2 mL | 1 x 10^8 cells/mL<br>0.5 - 6 mL |
| 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<br>(e.g. Corning Catalog #352058) | 14 mL (17 x 100 mm) polystyrene round-bottom tube<br>(e.g. Corning Catalog #352057) |
| 4 | Add Enrichment Cocktail to sample. | 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 centrifuging. | 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 mL |
| 6 | Add Biotin Selection Cocktail to sample. | 60 µL/mL of sample | 60 µL/mL of sample |
| | Mix and incubate. | 2 - 8°C for 15 minutes | 2 - 8°C for 15 minutes |
| 7 | Vortex Magnetic Particles.<br>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 2.5 mL for samples ≤ 2 mL<br>• 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 |
| 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™ EasySep™ Mouse Monocyte Enrichment Kit Protocol**

| STEP | INSTRUCTIONS | RoboSep™ (Catalog #20000 and #21000)  |
|---|---|---|
| 1 | Prepare sample at the indicated cell concentration within the volume range. | 1 x 10^8 cells/mL<br>0.5 - 6 mL |
| 2 | Add Rat Serum to sample. | 50 µL/mL of sample |
| 3 | Add sample to required tube. | 14 mL (17 x 100 mm) polystyrene round-bottom tube<br>(e.g. Corning Catalog #352057) |
| 4 | Add Enrichment Cocktail to sample. | 50 µL/mL of sample |
| | Incubate. | 2 - 8°C for 15 minutes |
| 5 | Wash the cells by topping up the sample tube with recommended medium and centrifuging. | 300 x g for 10 minutes |
| | Discard the supernatant and resuspend cells in the original volume with recommended medium. | 0.5 - 6 mL |
| 6 | Select protocol. | Mouse Monocyte Negative Selection 19761 |
| 7 | Vortex Magnetic Particles.<br>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. | Isolated cells are ready for use |

## Notes and Tips

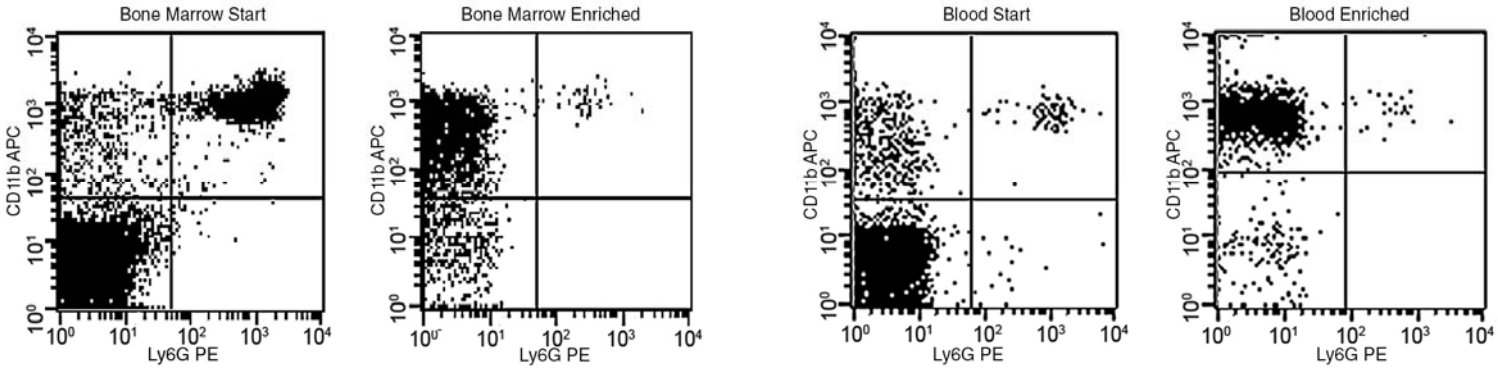
ASSESSING PURITY

To date, an exclusive marker for mouse bone marrow and peripheral blood monocytes has not been identified. However, monocytes are known to express CD11b, F4/80, CD115 (M-CSFR), Gr-1, and Ly-6C.

For purity assessment of monocytes (CD11b+Ly-6G-) by flow cytometry use the following fluorochrome-conjugated antibody clones:

- 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 peripheral blood, the monocyte content (CD11b+Ly-6G-) of the enriched fraction typically ranges from 92 - 98% (blood) and 80 - 93% (bone marrow). In the above examples, the purities of the start and final enriched fractions are 8.8% and 88.8% (bone marrow), and 9.7% and 95.1% (blood), respectively.

NOTE: RBCs were removed by lysis prior to flow cytometry.