

# EasySep™ Mouse Monocyte Isolation Kit



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

Catalog #19861

Negative Selection

Document #1000005282 | Version 02

## Description

Isolate untouched and highly purified monocytes from mouse bone marrow, splenocytes, whole blood, or other single-cell suspensions in as little as 15 minutes by immunomagnetic negative selection.

- Fast and easy-to-use
- Up to 95% purity
- No columns required
- Untouched, viable cells

This kit targets non-monocytes for removal with antibodies recognizing cell surface markers. Unwanted cells are labeled with antibodies and magnetic particles, and separated without columns using an EasySep™ magnet. Desired cells are simply poured or pipetted 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 Monocyte Isolation Cocktail Component A	19861CA	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™ Mouse Monocyte Isolation Cocktail Component B	19861CB	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™ Dextran RapidSpheres™ 50103	50103	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 water.
Normal Rat Serum	13551	1 x 2 mL	Store at -20°C.	Stable until expiry date (EXP) on label.	Mycoplasma-free normal rat serum.
RoboSep™ Empty Vial	27401	1	Not applicable	Not applicable	Not applicable

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.

## Additional Reagent Stability Information

REAGENT NAME	STORAGE	SHELF LIFE
Selection Cocktail (combined Component A + Component B)	Store at 2 - 8°C. Do not freeze.	Stable for up to 4 weeks. Do not exceed expiry date (EXP) of individual components.
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 6 minutes and resuspend cells at  $1 \times 10^8$  nucleated cells/mL in recommended medium.

Ammonium chloride treatment is not recommended when preparing bone marrow 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  $1 \times 10^8$  nucleated cells/mL in recommended medium. If there are less than  $5 \times 10^7$  nucleated cells/mL, resuspend in 500 µL of recommended medium.

### SPLEEN

Disrupt spleen in PBS 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  $1 \times 10^8$  nucleated cells/mL in recommended medium.

Ammonium chloride treatment is not recommended when preparing the splenocytes 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> and Mg<sup>++</sup>.

## Directions for Use – Manual EasySep™ Protocols

See page 2 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 Monocyte 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 0.5 - 8 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 (e.g. Catalog #38007)	14 mL (17 x 95 mm) polystyrene round-bottom tube (e.g. Catalog #38008)
4	Prepare Selection Cocktail in a tube. For each 1 mL of sample, prepare 100 µL of cocktail (50 µL of Component A + 50 µL of Component B).	Mix equal volumes of Component A and Component B. Selection Cocktail is stable at 2 - 8°C for up to 4 weeks.	Mix equal volumes of Component A and Component B. Selection Cocktail is stable at 2 - 8°C for up to 4 weeks.
	Mix and incubate.		
5	Add Selection Cocktail to sample. NOTE: Do not vortex cocktail.	100 µL/mL of sample	100 µL/mL of sample
	Mix and incubate.	2 - 8°C for 5 minutes	2 - 8°C for 5 minutes
6	Vortex RapidSpheres™. NOTE: Particles should appear evenly dispersed.	30 seconds	30 seconds
7	Add RapidSpheres™ to sample.	75 µL/mL of sample	75 µL/mL of sample
	Mix and incubate.	2 - 8°C for 3 minutes	2 - 8°C for 3 minutes
8	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 2.5 mL for samples &lt; 2 mL</li> <li>• Top up to 10 mL for samples ≥ 2 mL</li> </ul>
	Place the tube (without lid) into the magnet and incubate.	RT for 3 minutes	RT for 3 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.	Use a new 5 mL tube	Use a new 14 mL tube
10	Remove the tube from the magnet; Place the new tube (without lid) into the magnet and incubate for a second separation.	RT for 3 minutes	RT for 3 minutes
11	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.

Table 2. EasySep™ Mouse Monocyte 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 0.5 - 8 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 (e.g. Catalog #38007)	14 mL (17 x 95 mm) polystyrene round-bottom tube (e.g. Catalog #38008)
4	Prepare Selection Cocktail in a tube. For each 1 mL of sample, prepare 100 µL of cocktail (50 µL of Component A + 50 µL of Component B).	Mix equal volumes of Component A and Component B. Selection Cocktail is stable at 2 - 8°C for up to 4 weeks.	Mix equal volumes of Component A and Component B. Selection Cocktail is stable at 2 - 8°C for up to 4 weeks.
	Mix and incubate.	RT for 5 minutes	RT for 5 minutes
5	Add Selection Cocktail to sample. NOTE: Do not vortex cocktail.	100 µL/mL of sample	100 µL/mL of sample
	Mix and incubate.	2 - 8°C for 5 minutes	2 - 8°C for 5 minutes
6	Vortex RapidSpheres™. NOTE: Particles should appear evenly dispersed.	30 seconds	30 seconds
7	Add RapidSpheres™ to sample.	100 µL/mL of sample	100 µL/mL of sample
	Mix and incubate.	2 - 8°C for 3 minutes	2 - 8°C for 3 minutes
8	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 2.5 mL for samples &lt; 2 mL</li> <li>• Top up to 10 mL for samples ≥ 2 mL</li> </ul>
	Place the tube (without lid) into the magnet and incubate.	RT for 5 minutes	RT for 5 minutes
9	Carefully pipette** (do not pour) the enriched cell suspension into a new tube.	Use a new 5 mL tube	Use a new 14 mL tube
10	Remove the tube from the magnet; Place the new tube (without lid) into the magnet and incubate for another separation.	RT for 5 minutes	RT for 5 minutes
11	Repeat steps as indicated.	---	Steps 9 and 10 (for a total of 3 x 5-minute separations)
12	Carefully pipette** (do not pour) 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)

\*\* 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 2 for Sample Preparation and Recommended Medium. Refer to Table 3 for detailed instructions regarding the RoboSep™ procedure.

**Table 3. RoboSep™ Mouse Monocyte 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 0.5 - 8 mL	
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	Prepare Selection Cocktail in the RoboSep™ Empty Vial provided. See Table 4 for required volumes. NOTE: Do not vortex cocktail.	Mix equal volumes of Component A and Component B (see Table 4). Selection Cocktail is stable at 2 - 8°C for up to 4 weeks.	
	Mix and incubate.		
5	Select protocol.	Mouse Monocyte Isolation Kit 19861	
6	Vortex RapidSpheres™. NOTE: Particles should appear evenly dispersed.	30 seconds	
7	Load the carousel.	Follow on-screen prompts	
	Start the protocol.	Press the green "Run" button	
8	Unload the carousel when the run is complete.	Isolated cells are ready for use	

**Table 4. RoboSep™ Selection Cocktail Preparation**

START SAMPLE	COMPONENT A	COMPONENT B	SELECTION COCKTAIL TOTAL VOLUME
0.5 mL	75 µL	75 µL	150 µL
1 mL	100 µL	100 µL	200 µL
1.5 mL	125 µL	125 µL	250 µL
2 mL	150 µL	150 µL	300 µL
3 mL	200 µL	200 µL	400 µL
4 mL	250 µL	250 µL	500 µL
5 mL	300 µL	300 µL	600 µL
6 mL	350 µL	350 µL	700 µL
7 mL	400 µL	400 µL	800 µL
8 mL	450 µL	450 µL	900 µL

NOTE: RoboSep™ requires an extra 100 µL of the Selection Cocktail to run properly (compared to manual protocols).

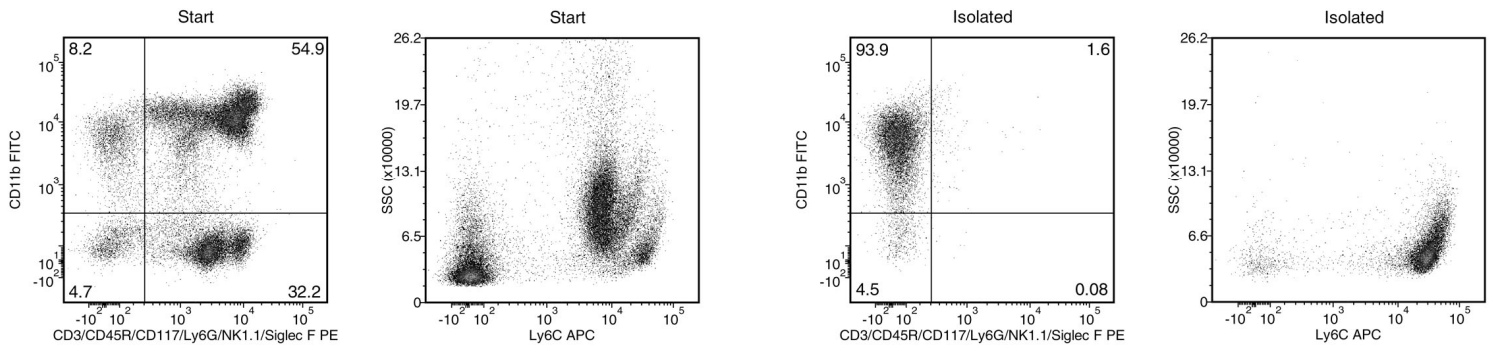
## Notes and Tips

### ASSESSING PURITY

To date, an exclusive marker for mouse monocytes has not been identified. However, monocytes are known to express CD11b and CD115 (M-CSFR), but not Ly-6G. Ly-6C expression is variable. For purity assessment by flow cytometry, use the following fluorochrome-conjugated antibody clones:

- Anti-Mouse CD11b Antibody, Clone M1/70 (Catalog #60001), and
- Anti-Mouse CD3e Antibody, Clone 145-2C11 (Catalog #60015), and
- Anti-Mouse CD45R (B220) Antibody, Clone RA3-6B2 (Catalog #60019), and
- Anti-Mouse Ly-6G Antibody, Clone 1A8 (Catalog #60031), and
- Anti-Mouse NK1.1 (CD161) Antibody, Clone PK136 (Catalog #60103), and
- Anti-mouse CD117 (c-Kit) antibody, clone ACK45, and
- Anti-mouse Siglec F antibody, clone E50-2440

## Data



Starting with bone marrow cells, the monocyte content (CD11b<sup>+</sup>/CD3<sup>-</sup>/CD45R<sup>-</sup>/CD117<sup>-</sup>/Ly-6G<sup>-</sup>/NK1.1<sup>-</sup>/Siglec F<sup>-</sup>/SSC low) of the isolated fraction is typically  $94.2 \pm 1.5\%$  (mean  $\pm$  SD using the purple EasySep™ Magnet).

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