EasySep™ Mouse Monocyte Isolation Kit

Negative Selection

Catalog #19861

For labeling up to 1 x 10e9 cells

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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

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

FROM BONE MARROW

Harvest bone marrow by preferred method. Crush bones in recommended medium using a mortar and pestle. Alternatively, 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. With either method, remove remaining clumps of cells and debris by passing cell suspension through a 70 µm mesh nylon strainer. Centrifuge at 300 x g for 6 minutes, discard supernatant and resuspend cells at 1 x 10e8 cells/mL in recommended medium.

FROM 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 10e8 cells/mL in recommended medium. If there are less than 5 x 10e7 cells/mL, resuspend in 500 µL of recommended medium.

FOR SPLEEN

Disrupt spleen in phosphate-buffered saline (PBS) containing 2% fetal bovine serum (FBS). Remove clumps 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 10e8 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. Hanks' Balanced Salt Solution (HBSS; 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 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 10e8 cells/mL 0.5 - 2 mL	1 x 10e8 cells/mL 0.5 - 8 mL
2	Add Normal 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. Corning® Catalog #352058)	14 mL (17 x 100 mm) polystyrene round-bottom tube (e.g. Corning® Catalog #352057)
4	Prepare Selection Cocktail in a tube. For each 1 mL of sample make 100 µL of cocktail (50 µl of Component A + 50 µL of Component B).	Mix equal volumes of Component A and Component B. Prepared cocktail is stable at 2 - 8°C for up to 4 weeks.	Mix equal volumes of Component A and Component B. Prepared 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.	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™.	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	• Top up to 2.5 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 3 minutes
9	Pick up the magnet, and in one continuous motion invert the magnet and tube, pouring off 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 and 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 off the enriched cell suspension* into a new tube.	Isolated cells are now ready for use	Isolated cells are now 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 10e8 cells/mL 0.5 - 2 mL	1 x 10e8 cells/mL 0.5 - 8 mL
2	Add Normal 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. Corning® Catalog #352058)	14 mL (17 x 100 mm) polystyrene round-bottom tube (e.g. Corning® Catalog #352057)
4	Prepare Selection Cocktail in a tube. For each 1 mL of sample make 100 µL of cocktail (50 µl of Component A + 50 µL of Component B).	Mix equal volumes of Component A and Component B. Prepared cocktail is stable at 2 - 8°C for up to 4 weeks.	Mix equal volumes of Component A and Component B. Prepared 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.	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™.	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	• Top up to 2.5 mL for samples < 2 mL • Top up to 10 mL for samples ≥ 2 mL
	Place the plasticware (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 new plasticware.	Use a new 5 mL tube	Use a new 14 mL tube
10	Remove the plasticware from the magnet and place the new plasticware (without lid) into the magnet and incubate for a second separation.	RT for 5 minutes	RT for 5 minutes
11	Repeat steps as indicated.	---	Steps 8 and 9 (for a total of 3 x 5-minute separations)
12	Carefully pipette (do not pour) the enriched cell suspension** into new plasticware.	Isolated cells are now ready for use	Isolated cells are now ready for use

RT - room temperature (15 - 25°C)
** Collect the entire supernatant, all at once, into a single pipette (e.g. for the EasyEights™ 5 mL tube use a 2 mL serological pipette and for the EasyEights™ 14 mL tube use a 10 mL serological pipette).

Directions for Use – Fully Automated RoboSep™ Protocol

See page 1 for Sample Preparation and Recommended Medium. Refer to Table 3 for detailed instructions regarding the RoboSep™ procedure.

Table 3. RoboSep™ Mouse Monocyte Isolaton Kit Protocol

STEP	INSTRUCTIONS	RoboSep™ (Catalog #20000 and #21000) 
1	Prepare sample at the indicated cell concentration within the volume range.	1 x 10e8 cells/mL 0.5 - 8 mL
2	Add Normal 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 (e.g. Corning® Catalog #352057)
4	Prepare Selection Cocktail in the RoboSep™ Empty Vial provided. See Table 4 for required volumes.	Mix equal volumes of Component A and Component B (see Table 4). Prepared cocktail is stable at 2 - 8°C for up to 4 weeks.
	Mix and incubate.	RT for 5 minutes
5	Select protocol.	Mouse Monocyte Isolation Kit 19861
6	Vortex RapidSpheres™.	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 now 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).

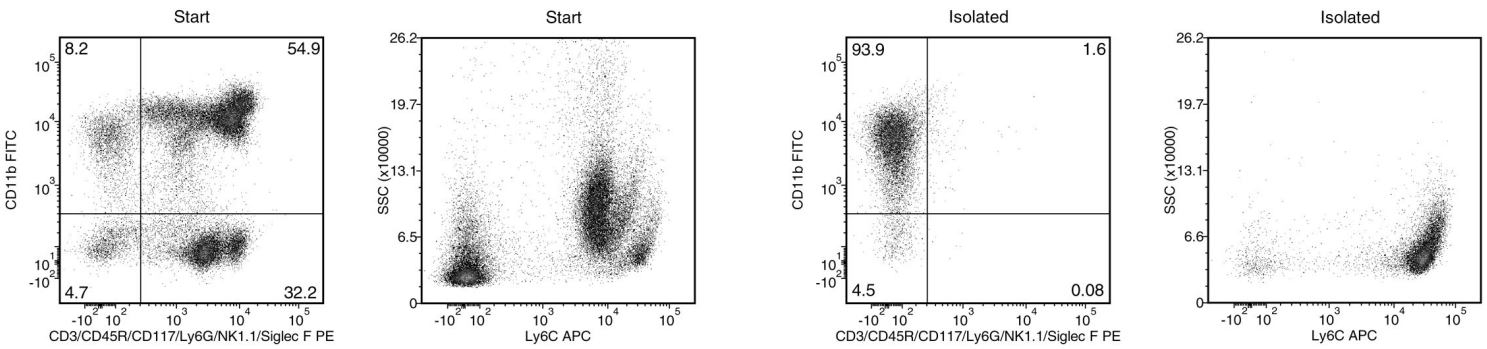
Notes and Tips

ASSESSING PURITY

To date, an exclusive marker for mouse monocytes has not been identified. However, monocytes are known to express CD11b, 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)
- Anti-Mouse CD3e Antibody, Clone 145-2C11 (Catalog #60015)
- Anti-Mouse CD45R (B220) Antibody, Clone RA3-6B2 (Catalog #60019)
- Anti-Mouse Ly-6G Antibody, Clone 1A8 (Catalog #60031)
- Anti-Mouse NK1.1 Antibody, Clone PK136 (Catalog #60103)
- anti-mouse CD117 (c-Kit) antibody, clone ACK45
- anti-mouse Siglec F antibody, clone E50-2440

Data



Starting with bone marrow cells, the monocyte content (CD11b+/CD3e-/CD45R-/CD117-/Ly-6G-/NK1.1-/Siglec F-/SSC low) of the isolated fraction is typically 94.2 ± 1.5% (mean ± SD using the purple EasySep™ Magnet).