EasySep™ Mouse F4/80 Positive Selection Kit

For processing 6 x 10^8 cells from peritoneal lavage

Catalog #100-0659

Positive Selection

Document #10000010447 | Version 01

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Description

Isolate highly purified F4/80+ cells from mouse peritoneal lavage by positive selection.

- Fast, easy-to-use, and column-free
- Up to 97% purity
- Isolated cells are not fluorochrome-labeled

This kit targets F4/80+ cells for positive selection with antibodies recognizing the F4/80 cell surface marker. Desired cells are labeled with antibodies and magnetic particles and separated without columns using an EasySep™ magnet. Unwanted cells are simply poured off while desired cells remain in the tube. Isolated cells are immediately available for downstream applications such as flow cytometry, cell culture, and cell-based experiments.

NOTE: This is the Product Information Sheet (PIS) for isolating F4/80+ cells from mouse peritoneal lavage. If isolating F4/80+ cells from mouse spleen or lung tissues, refer to the applicable PIS, available at www.stemcell.com, or contact us to request a copy.

Component Descriptions

COMPONENT NAME	COMPONENT #	QUANTITY	STORAGE	SHELF LIFE	FORMAT
EasySep™ Mouse F4/80 Positive Selection Component A	300-0251	1 x 0.3 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 F4/80 Positive Selection Component B	300-0253	1 x 0.6 mL	Store at 2 - 8°C. Do not freeze.	Stable until expiry date (EXP) on label.	A combination of monoclonal antibodies in PBS with 5% HPCD.
EasySep™ Dextran RapidSpheres™ 50100	50100	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 water.
Mouse FcR PolyBlock	300-0902	1 x 1.2 mL	Store at 2 - 8°C. Do not freeze.	Stable until expiry date (EXP) on label.	A combination of polyclonal antibodies and maltose in water with 5 µg/mL Triton X-100

BSA - bovine serum albumin; HPCD - 2-hydroxypropyl-β-cyclodextrin; PBS - phosphate-buffered saline

Components may be shipped at room temperature (15 - 25°C) but should be stored as indicated above.

Sample Preparation

PERITONEAL LAVAGE

1. Expose the abdominal cavity without puncturing peritoneum.
2. Use a 6 mL syringe and a 25 gauge needle to inject 5 mL of EasySep™ Buffer into the peritoneal cavity. Avoid puncturing internal organs.
3. Shake and invert the mouse several times to mix buffer throughout the peritoneal cavity.
4. Insert a needle and syringe into the cavity and collect liquid from the peritoneal cavity. Insert the needle as far away from intestines/fat deposits as possible to avoid the needle being blocked.
5. Collect cells into a 50 mL conical tube (e.g. Catalog #38010) or a smaller tube when processing fewer mice.
6. Centrifuge at 300 x g at room temperature (15 - 25°C) for 10 minutes with the brake on low. Carefully remove and discard the supernatant.
7. Resuspend cells at 2.5 x 10^7 nucleated cells/mL in recommended medium.

NOTE: Ammonium chloride treatment is not recommended when preparing the cells for separation.

SPLEEN OR LUNG TISSUE

If processing mouse spleen or lung tissue, refer to the applicable PIS, available at www.stemcell.com, or contact us to request a copy.

Recommended Medium

EasySep™ Buffer (Catalog #20144) or PBS containing 2% fetal bovine serum (FBS) and 1 mM EDTA. 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 F4/80 Positive Selection Kit Protocol for PERITONEAL LAVAGE

		EASYSEP™ MAGNETS	
STEP	INSTRUCTIONS	 **EasySep™ (Catalog #18000)**	**"The Big Easy" (Catalog #18001)** 
1	**Prepare sample at the indicated cell concentration within the volume range.**	2.5 x 10^7 cells/mL 0.1 - 2 mL	2.5 x 10^7 cells/mL 0.5 - 8 mL
2	**Add Mouse FcR PolyBlock 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 make 25 µL of cocktail (12.5 µL of Component A + 12.5 µL of Component B).**	Mix equal volumes of Component A and Component B. NOTE: Selection Cocktail must be prepared fresh before each use.	Mix equal volumes of Component A and Component B. NOTE: Selection Cocktail must be prepared fresh before each use.
	Incubate.	RT for 5 minutes	RT for 5 minutes
5	**Add Selection Cocktail to sample.** **NOTE: Do not vortex cocktail.**	25 µL/mL of sample	25 µL/mL of sample
	Mix and incubate.	RT for 5 minutes	RT for 5 minutes
6	**Vortex RapidSpheres™.** **NOTE: Particles should appear evenly dispersed.**	30 seconds	30 seconds
7	**Add RapidSpheres™ to sample.**	60 µL/mL of sample	60 µL/mL of sample
	Mix and incubate.	RT for 5 minutes	RT for 5 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 5 mL for samples ≤ 3 mL • Top up to 10 mL for samples > 3 mL
	Place the tube (without lid) into the magnet and incubate.	RT for 3 minutes	RT for 5 minutes
9	**Pick up the magnet, and in one continuous motion invert the magnet and tube,* pouring off the supernatant. Remove the tube from the magnet; this tube contains the isolated cells.**	Discard supernatant	Discard supernatant
10	**Repeat steps as indicated.**	Steps 8 and 9, two more times (total of 3 x 3-minute separations)	Steps 8 and 9, two more times (total of 3 x 5-minute separations)
11	**Resuspend cells in desired medium. Be sure to collect cells from the sides of the 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 F4/80 Positive Selection Kit Protocol for PERITONEAL LAVAGE

		EASYSEP™ MAGNETS		
STEP	INSTRUCTIONS	 EasyPlate™ (Catalog #18102)	 EasyEights™ (Catalog #18103) 5 mL tube	 EasyEights™ (Catalog #18103) 14 mL tube
1	Prepare sample at the indicated cell concentration within the volume range.	2.5 x 10^7 cells/mL 0.05 - 0.2 mL	2.5 x 10^7 cells/mL 0.25 - 2 mL	2.5 x 10^7 cells/mL 1 - 8 mL
2	Add Mouse FcR PolyBlock to sample.	50 µL/mL of sample	50 µL/mL of sample	50 µL/mL of sample
3	Add sample to required tube or plate.	Round-bottom, non-tissue culture-treated 96-well plate (e.g. Catalog #38018)	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 make 25 µL of cocktail (12.5 µL of Component A + 12.5 µL of Component B).	Mix equal volumes of Component A and Component B. NOTE: Selection Cocktail must be prepared fresh before each use.	Mix equal volumes of Component A and Component B. NOTE: Selection Cocktail must be prepared fresh before each use.	Mix equal volumes of Component A and Component B. NOTE: Selection Cocktail must be prepared fresh before each use.
	Incubate.	RT for 5 minutes	RT for 5 minutes	RT for 5 minutes
5	Add Selection Cocktail to sample. NOTE: Do not vortex cocktail.	25 µL/mL of sample	25 µL/mL of sample	25 µL/mL of sample
	Mix and incubate.	RT for 5 minutes	RT for 5 minutes	RT for 5 minutes
6	Vortex RapidSpheres™. NOTE: Particles should appear evenly dispersed.	30 seconds	30 seconds	30 seconds
7	Add RapidSpheres™ to sample.	60 µL/mL of sample	80 µL/mL of sample	80 µL/mL of sample
	Mix and incubate.	RT for 5 minutes	RT for 5 minutes	RT for 5 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 0.25 mL	Top up to 2.5 mL	• Top up to 5 mL for samples ≤ 3 mL • Top up to 10 mL for samples > 3 mL
	Place the tube or plate (without lid) into the magnet and incubate.	RT for 5 minutes	RT for 10 minutes	RT for 10 minutes
9	Carefully pipette* (do not pour) off the supernatant. Remove the tube or plate from the magnet; this tube or plate contains the isolated cells.	Discard supernatant	Discard supernatant	Discard supernatant
10	Add recommended medium to top up sample to the indicated volume. Mix by gently pipetting up and down 2 - 3 times.	Top up to 0.25 mL	Top up to 2.5 mL	• Top up to 5 mL for samples ≤ 3 mL • Top up to 10 mL for samples > 3 mL
	Place the tube or plate (without lid) into the magnet and incubate.	RT for 5 minutes	RT for 5 minutes	RT for 5 minutes
11	Carefully pipette* (do not pour) off the supernatant. Remove the tube or plate from the magnet; this tube or plate contains the isolated cells.	Discard supernatant	Discard supernatant	Discard supernatant
12	Repeat steps as indicated.	Steps 10 and 11 (total of 3 x 5-minute separations)	Steps 10 and 11 (total of 1 x 10-minute and 2 x 5-minute separations)	Steps 10 and 11 (total of 1 x 10-minute and 2 x 5-minute separations)
13	Resuspend cells in desired medium. Be sure to collect cells from the sides of the tube or plate.	Isolated cells are ready for use	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]).

Notes and Tips

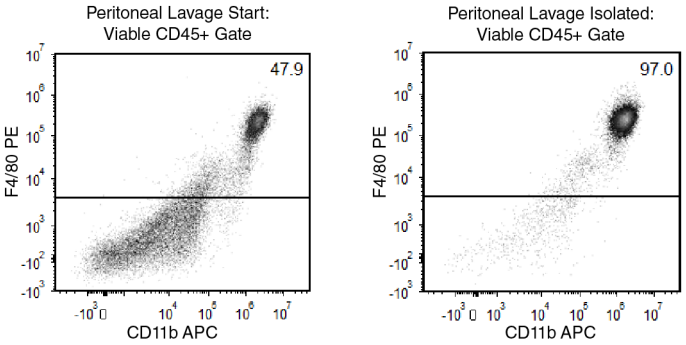
ASSESSING PURITY

For purity assessment by flow cytometry, use the following fluorochrome-conjugated antibody clones:

- Anti-Mouse F4/80 Antibody, Clone BM8 (Catalog #60027), and
- Anti-Mouse CD11b Antibody, Clone M1/70 (Catalog #100-0433), and
- Anti-Mouse CD45 Antibody, Clone 30-F11 (Catalog #60030)

NOTE: To exclude dead cells for purity assessment, use of the nuclear dye DRAQ7™ is recommended.

Data



Starting with lavage cells from the peritoneal cavity of a naïve mouse, the F4/80+ cell content of the isolated fraction is typically 97.0 ± 0.4% (mean ± SD using the purple EasySep™ magnet). In the above example, the purities of the start and final isolated fractions are 47.9% and 97.0%, respectively.