EasySep™ Mouse CD45 Positive Selection Kit

For processing 2 x 10^9 cells

Catalog #18945

Positive Selection

Document #10000005216 | Version 04

STEMCELL™ TECHNOLOGIES

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Description

Isolate highly purified CD45+ cells from mouse splenocytes, lungs, or other single-cell suspensions by immunomagnetic positive selection. When using single-cell suspensions from other tissue types, this kit may require optimization.

- Fast and easy-to-use
- Up to 98% purity
- No columns required
- Isolated cells are not fluorochrome-labeled

This kit targets CD45+ cells for positive selection with antibodies recognizing the CD45 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, culture, and cell-based experiments.

Component Descriptions

COMPONENT NAME	COMPONENT #	QUANTITY	STORAGE	SHELF LIFE	FORMAT
EasySep™ Mouse CD45 Positive Selection Kit Component A	18945CA	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 with 0.1% BSA.
EasySep™ Mouse CD45 Positive Selection Kit Component B	18945CB	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 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.

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

Components may be shipped at room temperature (15 - 25°C) and should be stored according to their storage conditions upon receipt.

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.

Sample Preparation

For automated and standardized tissue processing, see STEMprep™ Tissue Dissociator (Catalog #100-2112) at www.stemcell.com/stemprep. For manual processing, follow the steps below.

LUNG TISSUE

The following instructions are for processing 5 - 10 mouse lungs. If starting with more than 10 lungs, adjust volumes accordingly.

1. Prepare 10 mL of lung digestion medium by adding 0.25 mL of Liberase™ TM Research Grade (10 mg/mL; Sigma-Aldrich, Catalog #5401119001) and 1.5 mL of DNase I Solution (1 mg/mL; Catalog #07900) to 8.25 mL of RPMI 1640 Medium (Catalog #36750). Warm to room temperature (15 - 25°C).

   NOTE: 1 mL of Collagenase/Hyaluronidase (Catalog #07912) can also be used instead of Liberase™ TM Research Grade. If using Collagenase/Hyaluronidase to prepare lung digestion medium, decrease the volume of RPMI 1640 Medium to 7.5 mL.
2. Harvest lung tissue into a 50 mL conical tube with PBS containing 2% fetal bovine serum (FBS).
3. Transfer lung tissue to a new 50 mL conical tube containing 10 mL of digestion medium and mince the tissue into small pieces using scissors. Incubate at 37°C for 20 minutes on a shaking platform.
4. Place a 70 µm nylon mesh strainer (e.g. Catalog #27260) in a culture dish (e.g. Catalog #27100) and push the digested lung tissue through the strainer with the rubber end of a syringe plunger to obtain a cell suspension.
5. Place a new 70 µm nylon mesh strainer over a 50 mL conical tube and filter the cell suspension through the strainer. Rinse the strainer with recommended medium and collect in the same tube.
6. Centrifuge at 300 x g for 10 minutes at room temperature with the brake on low. Carefully remove and discard the supernatant.
7. Add 20 mL of Ammonium Chloride Solution (Catalog #07800) to the cell pellet. Incubate at room temperature for 5 minutes.
8. Top up to 50 mL with recommended medium. Centrifuge at 300 x g for 10 minutes at room temperature with the brake on low. Carefully remove and discard the supernatant.
9. Resuspend cells at 1 x 10^8 nucleated cells/mL in recommended medium.

SPLEEN

1. Harvest spleen into a conical tube with PBS containing 2% fetal bovine serum (FBS) or EasySep™ Buffer (Catalog #20144).
2. Place a 70 µm nylon mesh strainer (e.g. Catalog #27260) over a 50 mL conical tube (e.g Catalog #38010) and push spleen tissue through the strainer with the rubber end of a syringe plunger to obtain a single cell suspension.
3. Centrifuge at 300 x g for 10 minutes at room temperature with the brake on low. Carefully discard the supernatant.
4. Resuspend cells at 1 x 10^8 nucleated cells/mL in recommended medium.

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

Recommended Medium

EasySep™ Buffer (Catalog #20144), or PBS containing 2% FBS and 1 mM EDTA. Medium should be free of Ca++ and Mg++.

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 CD45 Positive Selection 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 0.1 - 1.5 mL	1 x 10^8 cells/mL 0.5 - 8 mL
	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)
2	**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.
3	**Add Selection Cocktail to sample.** **NOTE: Do not vortex cocktail.**	100 µL/mL of sample	100 µL/mL of sample
	Mix and incubate.	RT for 5 minutes	RT for 5 minutes
4	**Vortex RapidSpheres™.** **NOTE: Particles should appear evenly dispersed.**	30 seconds	30 seconds
5	**Add RapidSpheres™ to sample.**	50 µL/mL of sample	50 µL/mL of sample
	Mix and incubate.	RT for 3 minutes	RT for 3 minutes
6	**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 5 minutes	RT for 5 minutes
7	**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
8	**Repeat steps as indicated.**	Steps 6 and 7 (total of 2 x 5-minute separations)	Steps 6 and 7 (total of 2 x 5-minute separations)
9	**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 CD45 Positive Selection Kit Protocol

		EASYSEP™ MAGNETS	
STEP	INSTRUCTIONS	**EasyPlate™ (Catalog #18102)**	**EasyEights™ (Catalog #18103) 5 mL tube**
1	Prepare sample at the indicated cell concentration within the volume range.	1 x 10^8 cells/mL 0.05 - 0.15 mL	1 x 10^8 cells/mL 0.1 - 1.5 mL
	Add sample to required tube.	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)
2	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. 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.
3	Add Selection Cocktail to sample. NOTE: Do not vortex cocktail.	100 µL/mL of sample	100 µL/mL of sample
	Mix and incubate.	RT for 5 minutes	RT for 5 minutes
4	Vortex RapidSpheres™. NOTE: Particles should appear evenly dispersed.	30 seconds	30 seconds
5	Add RapidSpheres™ to sample.	50 µL/mL of sample	100 µL/mL of sample
	Mix and incubate.	RT for 3 minutes	RT for 3 minutes
6	Add recommended medium to top up sample to the indicated volume. Mix by gently pipetting up and down 2 - 3 times.	Top up to 250 µL	Top up to 2.5 mL
	Place the tube (without lid) into the magnet and incubate.	RT for 5 minutes	RT for 10 minutes
7	Carefully pipette** (do not pour) off the supernatant. Remove the tube from the magnet; this tube contains the isolated cells.	Discard supernatant	Discard supernatant
8	Repeat steps as indicated.	Steps 6 and 7 (total of 2 x 5-minute separations)	Steps 6 and 7 (total of 2 x 10-minute separations)
9	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)
** 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]).

Notes and Tips

ASSESSING PURITY

For purity assessment by flow cytometry, use one of the following antibody clones:

- Anti-Mouse CD45.2 Antibody, Clone 104 (Catalog #60118) for CD45.2 or Ly5.2 bearing mouse strains (e.g. A, AKR, BALB/c, CBA/Ca, CBA/J, C3H/He, C57BL, C57BR, C57L, C58, DBA/1, DBA/2, NZB, SWR, and 129)

OR

- Anti-Mouse CD45.1 Antibody, Clone A20 (Catalog #60117) for CD45.1 or Ly5.1 bearing mouse strains (e.g. RIII, SJL/J, STS/A, and DA)

Data

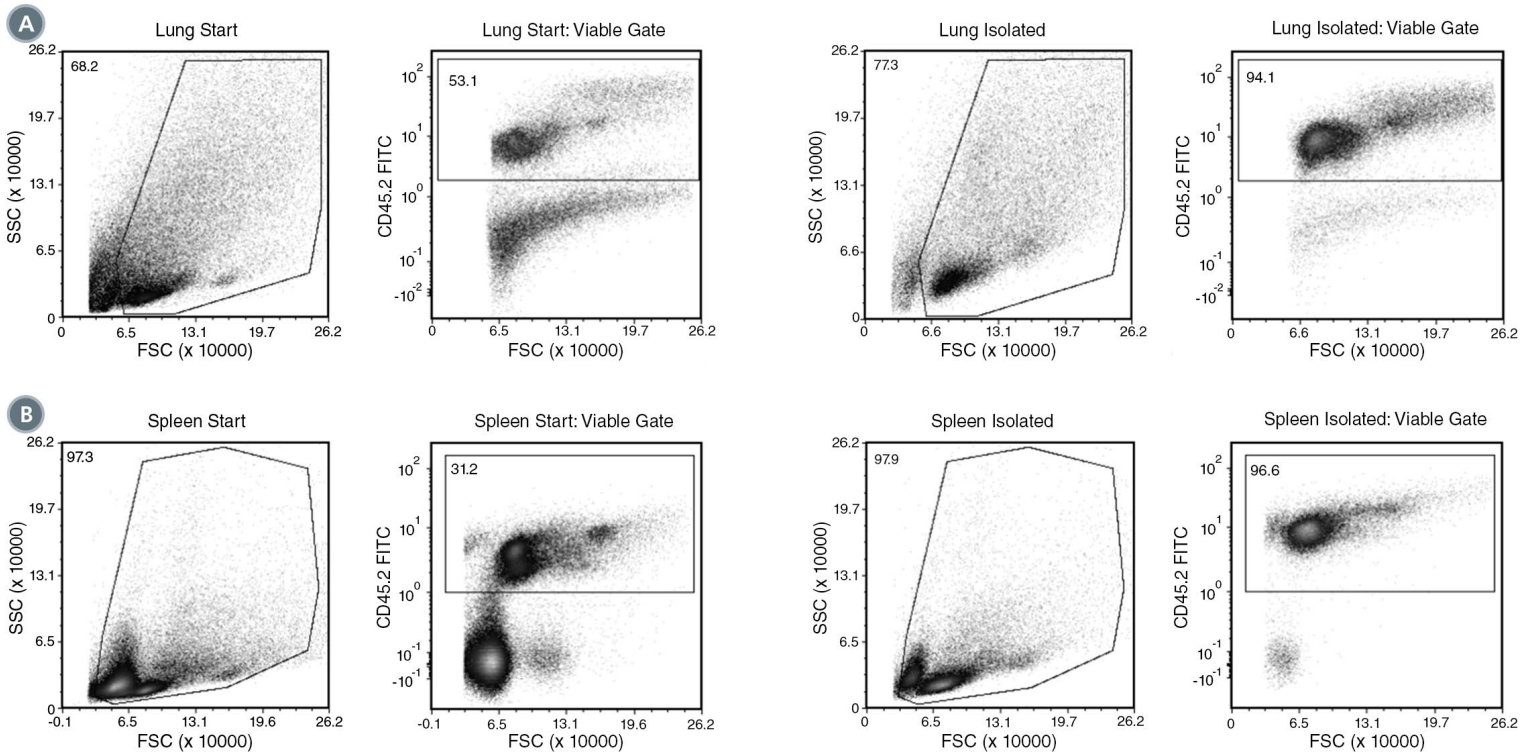

Figure 1. Isolation of CD45+ Cells from Naïve Mouse Lung Cells and Splenocytes

(A) Starting with a naïve mouse lung single-cell suspension, the leukocyte content (CD45+) of the isolated fraction is typically 97.0% ± 1.4% (mean ± SD using the purple EasySep™ Magnet). In the above example, the purities of the start and final isolated fractions in lung are 53.1% and 94.1%, respectively.

(B) Starting with unlysed naïve mouse splenocytes, the leukocyte content (CD45+) of the isolated fraction is typically 97.6 ± 1.3% (mean ± SD using the purple EasySep™ Magnet). In the above example using spleen, the purities of the start and final isolated fractions are 31.2% and 96.6%, respectively.

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