EasySep™ Mouse SCA1 Positive Selection Kit

Positive Selection

Catalog #18756

For processing 2 x 10^9 cells

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Description

Isolate highly purified SCA1+ cells from mouse bone marrow 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 97% purity
- No columns required

This kit targets SCA1+ cells for positive selection with an antibody recognizing the SCA1 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, or DNA/RNA extraction.

Component Descriptions

COMPONENT NAME	COMPONENT #	QUANTITY	STORAGE	SHELF LIFE	FORMAT
EasySep™ Mouse SCA1 PE Labeling Reagent	18756C.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, 0.1% BSA, and < 0.1% sodium azide. Includes an Fc receptor blocking antibody.
EasySep™ PE Selection Cocktail	18151	2 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™ Dextran RapidSpheres™ 50100	50100	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.

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.

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.

Recommended Medium

EasySep™ Buffer (Catalog #20144), RoboSep™ Buffer (Catalog #20104), 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 Table 1 for detailed instructions regarding the EasySep™ procedure for each magnet.

Table 1. EasySep™ Mouse SCA1 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 - 2 mL NOTE: If starting with fewer than 1 x 10^7 cells, resuspend cells in 0.1 mL.	1 x 10^8 cells/mL 0.25 - 8.5 mL NOTE: If starting with fewer than 2.5 x 10^7 cells, resuspend cells in 0.25 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	**Add Labeling Reagent to sample.**	50 µL/mL of sample	50 µL/mL of sample
	Mix and incubate.	RT for 15 minutes, protect from light	RT for 15 minutes, protect from light
3	**Add Selection Cocktail to sample.**	70 µL/mL of sample	70 µL/mL of sample
	Mix and incubate.	RT for 15 minutes, protect from light	RT for 15 minutes, protect from light
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 10 minutes, protect from light	RT for 10 minutes, protect from light
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 < 4 mL • Top up to 10 mL for samples ≥ 4 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, three more times (total of 4 x 5-minute separations)	Steps 6 and 7, three more times (total of 4 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.

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™ Mouse SCA1 Positive Selection 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 0.25 - 8.5 mL NOTE: If starting with fewer than 2.5 x 10^7 cells, resuspend cells in 0.25 mL.
	Add sample to required tube.	14 mL (17 x 95 mm) polystyrene round-bottom tube (e.g. Catalog #38008)
2	**Select protocol.**	Mouse SCA1 Positive Selection 18756-high purity
3	**Vortex RapidSpheres™.** **NOTE: Particles should appear evenly dispersed.**	30 seconds
4	**Load the carousel.**	Follow on-screen prompts
	Start the protocol.	Press the green “Run” button
5	**Unload the carousel when the run is complete. Remove the tube containing the isolated cells and resuspend in desired medium. Be sure to collect cells from the sides of the tube.**	Isolated cells are ready for use

Notes and Tips

ASSESSING PURITY

The positively selected cells have already been PE (phycoerythrin)-labeled so the purity can be assessed directly by flow cytometry.

If lineage-specific antigen labeling is desired, use the following fluorochrome-conjugated antibody clones:

- Anti-Mouse CD3e Antibody, Clone 145-2C11 (Catalog #60015), and
- Anti-Mouse CD11b Antibody, Clone M1/70 (Catalog #60001), and
- Anti-Mouse CD19 Antibody, Clone 6D5 (Catalog #60006), and
- Anti-Mouse CD45R Antibody, Clone RA3-6B2 (Catalog #60019), and
- Anti-Mouse Gr-1 Antibody, Clone RB6-8C5 (Catalog #60028), and
- Anti-Mouse TER119 Antibody, Clone TER-119 (Catalog #60033)

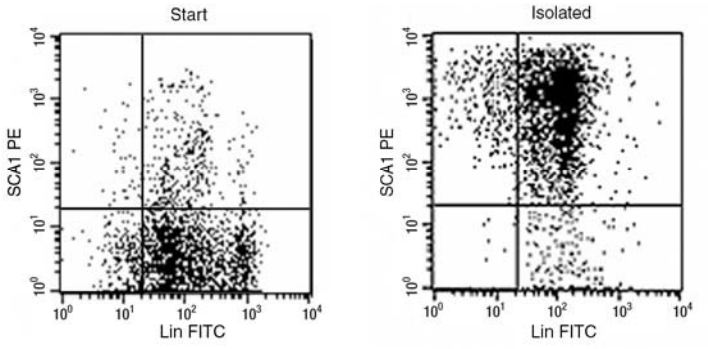
MOUSE STRAINS

Most hematopoietic stem and progenitor cells (HSPCs) from BALB/c and BALB/c-derived mouse strains do not express SCA1 (Spangrude & Brooks). Alternative markers should be used to select HSPCs from these mice.

TECHNICAL TIP

HSPCs and closely related primitive progenitor cells in mice are distinguished from the majority of the cells in hematopoietic tissues by their lack of expression markers specific to maturing blood cells (i.e. CD3, CD11b, CD45R (B220), Gr-1, TER119). In many mouse strains, HSPCs are positive for SCA1 (Ly-6A/E) and cKit (CD117) (Lin-SCA1+cKit+ phenotype; Spangrude et al.; Uchida & Weissman). More mature erythroid, myeloid, and megakaryocyte progenitor cells are also Lin- and cKit-, but negative for SCA1 (Lin-SCA1-cKit+ phenotype) (Akashi et al.). The various subsets can be significantly enriched by depletion of the lineage+ cells using EasySep™ Mouse Hematopoietic Progenitor Cell Isolation Kit (Catalog #19856), or by positive selection of SCA1+ or cKit+ cells with EasySep™.

Data



Starting with mouse bone marrow cells, the SCA1+ cell content of the isolated fraction typically ranges from 87 - 97%. In the above example, the purities of the start and final isolated fractions are 13.3% and 95.0%, respectively. HSPCs are present in the Lin-SCA1+cKIT+ population (see Notes and Tips for more information).

References

Akashi K et al. (2000) A clonogenic common myeloid progenitor that gives rise to all myeloid lineages. Nature 404(6774): 193–7.

Spangrude GJ et al. (1988) Purification and characterization of mouse hematopoietic stem cells. Science 241: 58–62.

Spangrude GJ & Brooks DM. (1993) Mouse strain variability in the expression of the hematopoietic stem cell antigen Ly-6A/E by bone marrow cells. Blood 82(11): 3327–32.

Uchida N & Weissman IL. (1992) Searching for hematopoietic stem cells: evidence that Thy-1.1lo Lin- Sca-1+ cells are the only stem cells in C57BL/Ka-Thy-1.1 bone marrow. J Exp Med 175(1): 175–84.