EasySep™

Negative Selection

Catalog #19756

EasySep™ Mouse Hematopoietic Progenitor Cell Enrichment Kit

For processing 1 x 10^9 cells

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Description

Isolate untouched and highly purified hematopoietic progenitor cells from mouse bone marrow by immunomagnetic negative selection. When using single-cell suspensions from other tissue types, this kit may require optimization.

- Fast, easy-to-use and column-free
- Up to 90% purity
- Isolated cells are untouched

This kit targets non-hematopoietic progenitor cells for removal with biotinylated antibodies recognizing specific cell surface markers. Unwanted cells are labeled with biotinylated antibodies and magnetic particles, and separated without columns using an EasySep™ magnet. Desired cells are simply poured off into a new 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 Hematopoietic Progenitor Cell Isolation Cocktail	19856C	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™ Biotin Selection Cocktail	19153	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.
EasySep™ M Prog Magnetic Microparticles	19350	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 TBS.
Normal Rat Serum	13551	1 x 2 mL	Store at -20°C.	Stable until expiry date (EXP) on label.	Mycoplasma-free normal rat serum.

BSA - bovine serum albumin; PBS - phosphate-buffered saline; TBS - TRIS-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
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 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. HBSS, Modified (Without Ca++ and Mg++; Catalog #37250) can be used in place of PBS. Medium should be free of Ca++, Mg++, and biotin.

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 Hematopoietic Progenitor Cell Enrichment 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 0.5 - 2 mL	1 x 10^8 cells/mL 0.5 - 8.5 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. Corning Catalog #352058)	14 mL (17 x 100 mm) polystyrene round-bottom tube (e.g. Corning Catalog #352057)
4	Add Isolation Cocktail to sample.	50 µL/mL of sample	50 µL/mL of sample
	Mix and incubate.	2 - 8°C for 15 minutes	2 - 8°C for 15 minutes
OPTIONAL WASH STEP NOTE: This will improve purity but may reduce recovery.		---	---
5	Wash the cells by topping up the sample tube with recommended medium and centrifuging.	300 x g for 10 minutes	300 x g for 10 minutes
	Discard the supernatant and resuspend cells in the original volume with recommended medium.	0.5 - 2 mL	0.5 - 8.5 mL
6	Add Biotin Selection Cocktail to sample.	100 µL/mL of sample	100 µL/mL of sample
	Mix and incubate.	2 - 8°C for 15 minutes	2 - 8°C for 15 minutes
7	Vortex Magnetic Particles. NOTE: Particles should appear evenly dispersed.	30 seconds	30 seconds
8	Add Magnetic Particles to sample.	50 µL/mL of sample*	50 µL/mL of sample*
	Mix and incubate.	2 - 8°C for 10 minutes	2 - 8°C for 10 minutes
9	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 3 minutes	RT for 3 minutes
10	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)

* Purity may be improved by adding Magnetic Particles at an increased concentration of 75 µL/mL of sample. NOTE: This will improve purity but may reduce recovery.

** 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 Hematopoietic Progenitor Cell Enrichment 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 0.5 - 8 mL
2	Add 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	Add Isolation Cocktail to sample	50 µL/mL of sample
	Mix and incubate.	2 - 8°C for 15 minutes
5	Wash the cells by topping up the sample tube with recommended medium and centrifuging.	300 x g for 10 minutes
	Discard the supernatant and resuspend cells in the original volume with recommended medium.	0.5 - 6.5 mL
6	Select protocol.	• Mouse Hematopoietic Progenitor Negative Selection 19756 - high purity • Mouse Hematopoietic Progenitor Negative Selection 19756 - high recovery
7	Vortex Magnetic Particles. NOTE: Particles should appear evenly dispersed.	30 seconds
8	Load the carousel.	Follow on-screen prompts
	Start the protocol.	Press the green “Run” button
9	Unload the carousel when the run is complete.	Isolated cells are ready for use

Notes and Tips

ASSESSING PURITY

The first step to isolation of mouse hematopoietic stem and progenitor cells (HSPCs) from bone marrow (BM) consists of removing mature cells that express 'lineage' (Lin) antigens specific to terminally differentiated blood cells. Lineage antigens are absent or weakly expressed on HSPCs. Lineage antigens include CD3, CD11b, CD19, CD45R (B220), Ly6G/C (Gr-1), and TER119.

In many mouse strains, HSPCs are positive for Sca1 (Ly-6A/E) and c-Kit (the receptor for SCF, also known as CD117) and referred to as LSK (Lin-Sca1+c-Kit+; Spangrude et al. 1988, Okada et al. 1992, Osawa et al. 1996, Uchida et al. 1992). LSK cells, which make up < 0.1% of nucleated BM cells, contain most repopulating stem cells but are depleted of more mature erythroid, myeloid, and megakaryocyte cells including most colony-forming units (CFUs) which are Lin-Sca1-/low c-Kit+ (Akashi et al. 2000). Mouse HSPCs are heterogeneous for other antigens such as CD34 and Thy1.

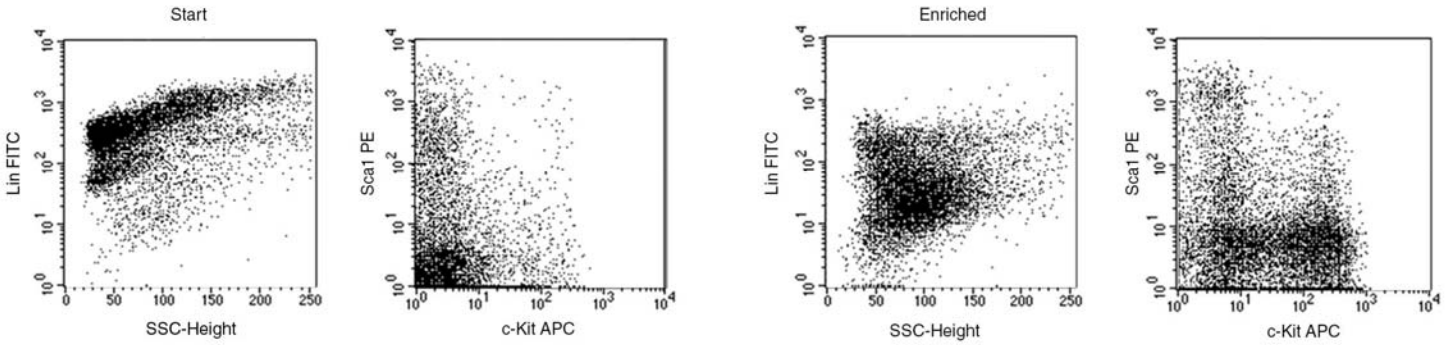
For purity assessment of these subsets of progenitor cells by flow cytometry use the following fluorochrome-conjugated antibody clones:

- Anti-Mouse CD117 Antibody, Clone 2B8 (Catalog #60025), and
- Anti-Mouse Sca1 Antibody, Clone E13-161.7 (Catalog #60032), and
- Anti-mouse lineage-specific antibodies (see below)

For lineage-specific antibody labeling use the folllowing 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)

Data



Starting with mouse bone marrow, the lineage-negative cell content of the enriched fraction is typically 60 - 90%. In the above example, the purities of the start and final enriched fractions are 10% and 72%, respectively.

References

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

Okada S et al. (1992) In vivo and in vitro stem cell function of c-kit and Sca-1-positive murine hematopoietic cells. Blood 80(12): 3044–50.

Osawa M et al. (1996) In vivo self-renewal of c-Kit+ Sca-1+ Lin(low/-) hemopoietic cells. J Immunol 156(9): 3207–14.

Spangrude GJ et al. (1988) Purification and characterization of mouse hematopoietic stem cells. Science 24(4861): 58–62.

Uchida N et al. (1992) Searching for hematopoietic stem cells: evidence that Thy-1.1lo Lin- Sca1+ cells are the only stem cells in C57BL/Ka-Thy-1.1 bone marrow. J Exp Med 175(1): 175–84.