EasySep™ Mouse Hematopoietic Progenitor Cell Isolation Kit

For processing 1 x 10^9 cells

Catalog #19856
Catalog #19856RF RoboSep™

Negative Selection

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Scientists Helping Scientists™ | WWW.STEMCELL.COM
TOLL FREE PHONE 1 800 667 0322 • PHONE +1 604 877 0713
INFO@STEMCELL.COM • TECHSUPPORT@STEMCELL.COM
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Description

Isolate untouched and highly purified 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 84% purity
- Untouched, viable cells

This kit targets non-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, and cell-based experiments.

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™ Streptavidin RapidSpheres™ 50001	50001	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 PBS.
EasySep™ Mouse FcR Blocker	18730	1 x 0.2 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.

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 aggregates by gently passing the cell suspension through the syringe several times. Alternatively, crush bones using a mortar and pestle. Remove remaining aggregates 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 nucleated cells/mL in recommended medium.

Ammonium chloride treatment is not recommended when preparing bone marrow for separation.

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++, 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 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 10^8 cells/mL 0.5 - 2 mL	1 x 10^8 cells/mL 0.5 - 8 mL
2	Add FcR Blocker to sample.	20 µL/mL of sample	20 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	Add Isolation Cocktail to sample. NOTE: Do not vortex cocktail.	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
5	Vortex RapidSpheres™. NOTE: Particles should appear evenly dispersed.	30 seconds	30 seconds
6	Add RapidSpheres™ to sample.	75 µL/mL of sample	75 µL/mL of sample
	Mix and incubate.	2 - 8°C for 10 minutes	2 - 8°C for 10 minutes
7	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
8	Pick up the magnet, and in one continuous motion invert the magnet and tube,* pouring the enriched cell suspension into a new tube.	Use a new 14 mL tube	• Use a new 14 mL tube for start samples ≤ 4 mL • Use a new 50 mL tube for start samples > 4 mL
9	Remove the tube from the magnet and add recommended medium to the indicated volume. Mix by gently pipetting up and down 5 - 6 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
10	Place the tube (without lid) into the magnet and incubate.	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.	Combine with first poured-off fraction from step 8 Isolated cells are ready for use	Combine with first poured-off fraction from step 8 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.

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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 Isolation Kit Protocol

STEP	INSTRUCTIONS	RoboSep™ (Catalog #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 FcR Blocker to sample.	20 µL/mL of sample
3	Add sample to required tube.	14 mL (17 x 95 mm) polystyrene round-bottom tube (e.g. Catalog #38008)
4	Select protocol.	Mouse Hematopoietic Progenitor Cell Isolation 19856
5	Vortex RapidSpheres™. NOTE: Particles should appear evenly dispersed.	30 seconds
6	Load the carousel.	Follow on-screen prompts
	Start the protocol.	Press the green “Run” button
7	Unload the carousel when the run is complete.	Isolated cells are ready for use

Notes and Tips

ASSESSING PURITY

The first step for isolation of mouse hematopoietic stem and progenitor cells (HSPCs) from bone marrow 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+).[1,2,3,4] 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 megakaryoid cells including most colony-forming units (CFUs) which are Lin-Sca1-/^{low}c-Kit+.[5] Mouse HSPCs are heterogeneous for other antigens such as CD34 and Thy1.

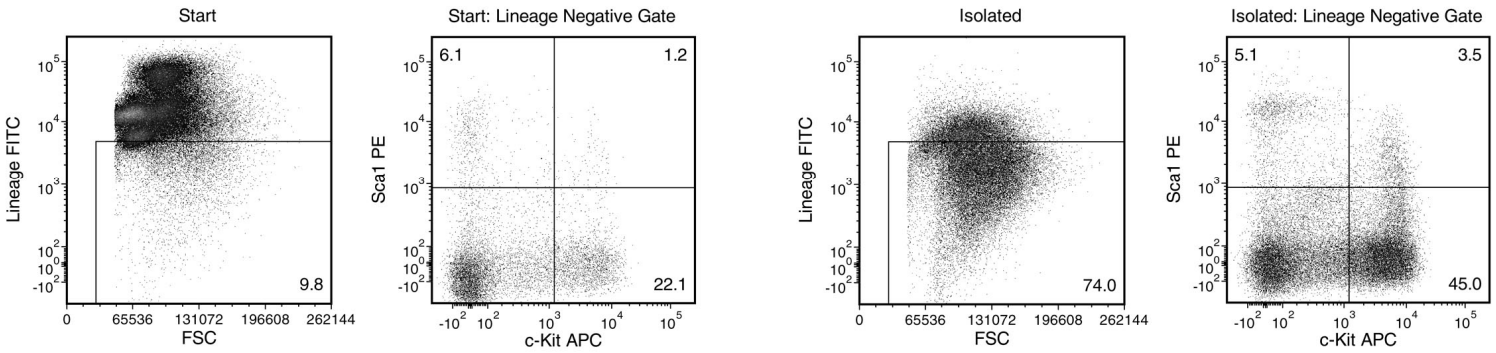
For purity assessment of these subsets of progenitor cells by flow cytometry, use the following fluorochrome-conjugated antibody clones:

- 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 antigen labeling, 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)

Data



Starting with a mouse bone marrow cell suspension, the lineage antigen-negative cell content of the isolated fraction typically ranges from 60 - 84%.

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References

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2. 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.
3. 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.
4. Osawa M et al. (1996) In vivo self-renewal of c-Kit+ Sca-1+ Lin(low/-) hemopoietic stem cells. J Immunol 156(9): 3207–14.
5. Akashi K et al. (2000) A clonogenic common myeloid progenitor that gives rise to all myeloid lineages. Nature 404(6774): 193–7.