EasySep™ Mouse CD93 (AA4.1) Positive Selection Kit

Positive Selection

Catalog #18762

For processing 2 x 10^9 cells

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Description

Isolate highly purified CD93+ (AA4.1) cells from mouse bone marrow or fetal liver 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 97% purity
- No columns required

This kit targets mouse CD93+ (AA4.1) cells for positive selection with antibodies recognizing the CD93 surface markers. 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 CD93 (AA4.1) Labeling Reagent	18762C	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™ Magnetic Nanoparticles Positive Selection	18150	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.

FETAL LIVER

Dissect fetal livers from mouse embryos. Resuspend to a single-cell suspension by pipetting cells up and down, then filter through a 70 µm mesh nylon strainer. Resuspend cells at 5 x 10^7 cells/mL.

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 CD93 (AA4.1) 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.**	• For bone marrow: 1 x 10^8 cells/mL • For fetal liver: 5 x 10^7 cells/mL 0.1 - 2 mL NOTE: If starting with fewer than 1 x 10^7 cells, resuspend in 0.1 mL	• For bone marrow: 1 x 10^8 cells/mL • For fetal liver: 5 x 10^7 cells/mL 0.25 - 8 mL NOTE: If starting with fewer than 2.5 x 10^7 cells, resuspend in 0.25 mL
	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)
2	**Add Labeling Reagent to sample.**	50 µL/mL cells	50 µL/mL cells
	Mix and incubate.	RT for 15 minutes, protect from light	RT for 15 minutes, protect from light
3	**Add PE Selection Cocktail.**	70 µL/mL cells	70 µL/mL cells
	Mix and incubate.	RT for 15 minutes, protect from light	RT for 15 minutes, protect from light
4	**Mix Magnetic Particles.** **NOTE: Particles should appear evenly dispersed.**	Pipette up and down more than 5 times	Pipette up and down more than 5 times
5	**Add Magnetic Particles to sample.**	50 µL/mL cells	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.**	• For bone marrow: Steps 6 and 7, two more times (for a total of 3 x 5-minute separations) • For fetal liver: Steps 6 and 7, three more times (for a total of 4 x 5-minute separations)	• For bone marrow: Steps 6 and 7, two more times (for a total of 3 x 5-minute separations) • For fetal liver: Steps 6 and 7, three more time (for a 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)

* Decreasing the top-up volume will increase cell recovery, but may slightly reduce cell purity.

** 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™ EasySep™ Mouse CD93 (AA4.1) Positive Selection Kit Protocol

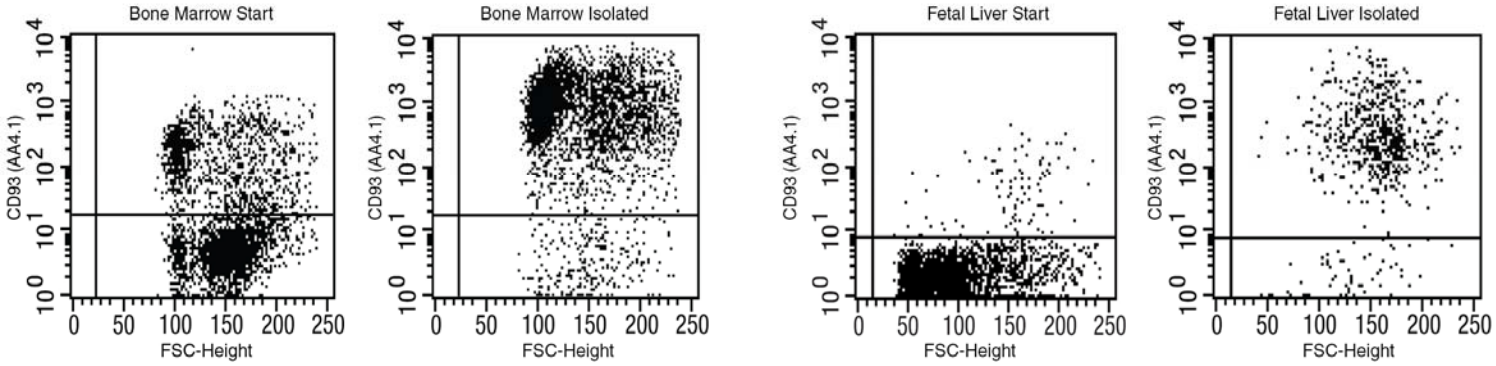
STEP	INSTRUCTIONS	RoboSep™ (Catalog #20000 and #21000) 
1	**Prepare sample at the indicated cell concentration within the volume range.**	• For bone marrow: 1 x 10^8 cells/mL • For fetal liver: 5 x 10^7 cells/mL 0.25 - 8.5 mL NOTE: If starting with fewer than 2.5 x 10^7 cells, resuspend in 0.25 mL
	Add sample to required tube.	14 mL (17 x 100 mm) polystyrene round-bottom tube (e.g. Corning Catalog #352057)
2	**Select protocol.**	Mouse CD93 Positive Selection 18762-high purity
3	**Mix Magnetic Particles.** **NOTE: Particles should appear evenly dispersed.**	Pipette up and down more than 5 times
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.**	Isolated cells are ready for use

Notes and Tips

ASSESSING PURITY

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

Data



Starting with mouse bone marrow or fetal liver cells, the CD93+ (AA4.1) content of the isolated fraction is typically 86.7 - 98.5% for bone marrow, or 74.4 - 97.0% for fetal liver. In the above examples, the purities of the start and final isolated fractions for bone marrow are 39.7% and 94.4%, respectively, and for fetal liver are 2.7% and 91.3%, respectively.