EasySep™ Mouse CD90.1 Positive Selection Kit

For processing 2 x 10^9 cells

Catalog #18958
#18958RF RoboSep™

Positive Selection

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Description

Isolate highly purified CD90.1+ (Thy1.1+) cells from mouse splenocytes, lymph nodes, or peripheral blood by immunomagnetic positive selection. This kit can also be used for the isolation of CD90.1+ cells from adoptive cell transfer (ACT) samples. 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
- Isolated cells are not fluorochrome-labeled

This kit targets CD90.1+ cells for positive selection with an antibody that recognizes the CD90.1 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 CD90.1 Positive Selection Cocktail	18958C	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 and 0.1% BSA.
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

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

SPLEEN or LYMPH NODE

Disrupt spleen or lymph node in recommended medium. Remove aggregates and debris by passing cell suspension through a 70 µm mesh nylon strainer (e.g. Catalog #27216). Centrifuge at 300 x g for 10 minutes and resuspend at 1 x 10^8 nucleated cells/mL in recommended medium.

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

PERIPHERAL BLOOD

Blood should be lysed prior to use. Mix 1 part blood with 9 parts Ammonium Chloride Solution (Catalog #07800) and incubate on ice for 15 minutes. Centrifuge at 300 x g for 6 minutes. Discard supernatant and wash cell pellet once with recommended medium. Discard supernatant and resuspend cell pellet at 1 x 10^8 cells/mL in recommended medium. If there are less than 5 x 10^7 cells/mL, resuspend in 500 µL of 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++ and Mg++.

Directions for Use – Manual EasySep™ Protocols

See page 1 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 CD90.1 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.**	1 x 10^8 cells/mL 0.25 - 2 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)
3	**Add Selection Cocktail to sample.** **NOTE: Do not vortex cocktail.**	50 µL/mL of sample For ACT samples: 25 µL/mL of sample	50 µL/mL of sample For ACT samples: 25 µL/mL of sample
	Mix and incubate.	RT for 3 minutes	RT for 3 minutes
4	**Vortex RapidSpheres™.** **NOTE: Particles should appear evenly dispersed.**	30 seconds	30 seconds
5	**Add RapidSpheres™ to sample.**	40 µL/mL of sample For ACT samples: 25 µL/mL of sample	40 µL/mL of sample For ACT samples: 25 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 ≤ 2 mL • Top up to 10 mL for samples > 2 mL
	Place the tube (without lid) into the magnet and incubate.	RT for 3 minutes	RT for 3 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, two more times (total of 3 x 3-minute separations)	Steps 6 and 7, two more times (total of 3 x 3-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

ACT - adoptive cell transfer; 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 CD90.1 Positive Selection Kit Protocol

		EASYSEP™ MAGNETS		
STEP	INSTRUCTIONS	**EasyPlate™ (Catalog #18102)**	**EasyEights™ (Catalog #18103)**	
			5 mL tube	**14 mL tube**
1	**Prepare sample at the indicated cell concentration within the volume range.**	1 x 10^8 cells/mL 0.1 - 0.2 mL	1 x 10^8 cells/mL 0.25 - 2 mL	1 x 10^8 cells/mL 0.5 - 8 mL
	Add sample to required tube (or plate when using the EasyPlate™ EasySep™ Magnet).	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)	14 mL (17 x 95 mm) polystyrene round-bottom tube (e.g. Catalog #38008)
3	**Add Selection Cocktail to sample.** **NOTE: Do not vortex cocktail.**	50 µL/mL of sample For ACT samples: 25 µL/mL of sample	50 µL/mL of sample For ACT samples: 25 µL/mL of sample	50 µL/mL of sample For ACT samples: 25 µL/mL of sample
	Mix and incubate.	RT for 3 minutes	RT for 3 minutes	RT for 3 minutes
4	**Vortex RapidSpheres™.** **NOTE: Particles should appear evenly dispersed.**	30 seconds	30 seconds	30 seconds
5	**Add RapidSpheres™ to sample.**	40 µL/mL of sample For ACT samples: 25 µL/mL of sample	40 µL/mL of sample For ACT samples: 25 µL/mL of sample	40 µL/mL of sample For ACT samples: 25 µL/mL of sample
	Mix and incubate.	RT for 3 minutes	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 0.25 mL	Top up to 2.5 mL	• Top up to 5 mL for samples ≤ 2 mL • Top up to 10 mL for samples > 2 mL
	Place the tube or plate (without lid) into the magnet and incubate.	RT for 3 minutes	RT for 10 minutes	RT for 10 minutes
7	**Carefully pipette** (do not pour) off the supernatant. Remove the tube or plate, containing the isolated cells, from the magnet.**	Discard supernatant	Discard supernatant	Discard supernatant
8	**Repeat steps as indicated.**	Steps 6 and 7, two more times (total of 3 x 3-minute separations)	Steps 6 and 7, two more times (total of 3 x 10-minute separations)	Steps 6 and 7, two more times (total of 3 x 10-minute separations)
8	**Resuspend cells in desired medium. Be sure to collect cells from the sides of the tube or plate.**	Isolated cells are ready for use	Isolated cells are ready for use	Isolated cells are ready for use

ACT - adoptive cell transfer; 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]; for EasyEights™ 14 mL tube, use a 10 mL serological pipette [Catalog #38004]).

Directions for Use – Fully Automated RoboSep™ Protocol

See page 1 for Sample Preparation and Recommended Medium. Refer to Table 3 for detailed instructions regarding the RoboSep™ procedure.

Table 3. RoboSep™ Mouse CD90.1 Positive Selection 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
	Add sample to required tube.	14 mL (17 x 95 mm) polystyrene round-bottom tube (e.g. Catalog #38008)
2	Select protocol.	Mouse CD90.1 (Thy-1.1) Positive Selection Kit 18958 For ACT: Mouse CD90.1 (Thy-1.1) Positive Selection Kit 18958 - ACT
3	Vortex RapidSpheres™. NOTE: Particles should appear evenly dispersed.	30 seconds
5	Load the carousel.	Follow on-screen prompts
	Start the protocol.	Press the green “Run” button
6	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

ACT - adoptive cell transfer

Notes and Tips

MOUSE STRAINS

This kit has been verified for use with FVB mouse strains and is expected to be compatible with other Thy1.1+ strains.

ASSESSING PURITY

For purity assessment of CD90.1+ cells by flow cytometry, use the following fluorochrome-conjugated antibody clone:

- Anti-Rat CD90 Antibody, Clone OX-7 (Catalog #60024)

Data

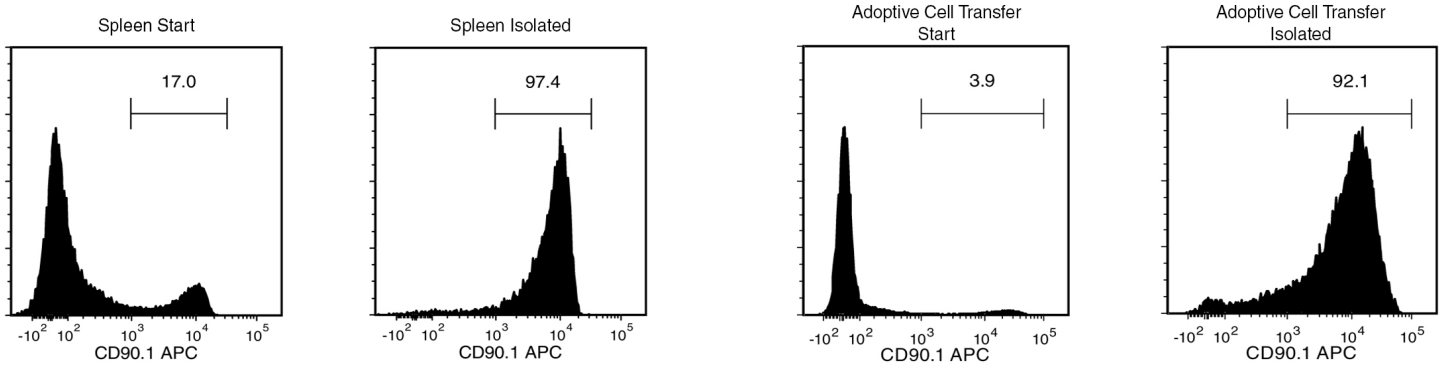

Figure 1. Isolation of CD90.1+ Cells from Mouse Splenocytes

Starting with mouse splenocytes, the CD90.1+ (Thy1.1+) cell content of the isolated fraction is typically 93.5 ± 3.9% (mean ± SD using the purple EasySep™ Magnet). In the above example, the purities of the start and final isolated fractions are 17.0% and 97.4%, respectively. Starting with mouse splenocytes from adoptive cell transfer samples, the CD90.1+ (Thy1.1+) cell content of the isolated fraction is typically 90.8 ± 1.5% (mean ± SD using the purple EasySep™ Magnet). In the above example, the purities of the start and final isolated fractions are 3.9% and 92.1%, respectively.

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