EasySep™ Mouse CD138 Positive Selection Kit

Positive Selection

Catalog #18957

For labeling 2 x 10^9 cells

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Description

Isolate highly purified CD138+ cells from single-cell suspensions of mouse splenocytes, lymph nodes or bone marrow by immunomagnetic positive selection. When using single-cell suspensions from other tissue types, this kit may require optimization.

- Fast, easy-to-use and column-free
- Up to 97% purity with immunized mice
- Useful for the enrichment of plasma cells
- Compatible with hybridoma generation protocols

This kit targets CD138+ cells for positive selection with an antibody recognizing the CD138 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, cell culture and hybridoma generation.

Component Descriptions

COMPONENT NAME	COMPONENT #	QUANTITY	STORAGE	SHELF LIFE	FORMAT
EasySep™ Mouse CD138 Positive Selection Cocktail	18957C	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 with 10% HPCD.
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.
EasySep™ Mouse FcR Blocker	18731	2 x 0.5 mL	Store at 2 - 8°C. Do not freeze.	Stable until expiry date (EXP) on label.	A monoclonal antibody in PBS, 0.1% BSA, and 0.1% sodium azide.

BSA - bovine serum albumin; HPCD - 2-hydroxypropyl-β-cyclodextrin; PBS - phosphate-buffered saline

Components may be shipped at room temperature (15 - 25°C) but should be stored as indicated above.

Please refer to the Safety Data Sheet (SDS) for hazard information.

Sample Preparation

SPLEEN or LYMPH NODE

Disrupt spleen or lymph node tissue in cold PBS or Hanks' Balanced Salt Solution containing 2% fetal bovine serum (FBS). Remove clumps and debris by passing cell suspension through a 70 µm mesh nylon strainer. Centrifuge at 300 x g for 10 minutes and resuspend at 1 x 10^8 nucleated cells/mL in cold recommended medium. Keep cells on ice until ready for use.

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

BONE MARROW

Flush bone marrow cells from femur and tibia into cold 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 cold recommended medium. Keep cells on ice until ready for use.

Recommended Medium

EasySep™ Buffer (Catalog #20144), RoboSep™ Buffer (Catalog #20104), or PBS containing 2% 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 Tables 1 and 2 for detailed instructions regarding the EasySep™ procedure for each magnet.

Table 1. EasySep™ Mouse CD138 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 and keep cold until use.**	1 x 10^8 cells/mL 0.5 - 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. Corning Catalog #352058)	14 mL (17 x 100 mm) polystyrene round-bottom tube (e.g. Corning Catalog #352057)
2	**Add FcR blocker to sample and mix.**	50 µL/mL of sample	50 µL/mL of sample
3	**Add Selection Cocktail to sample.**	50 µL/mL of sample	50 µL/mL of sample
	Mix and incubate.	On ice for 5 minutes	On ice for 5 minutes
4	**Vortex RapidSpheres™.**	30 seconds	30 seconds
5	**Add RapidSpheres™ to sample.**	50 µL/mL of sample	50 µL/mL of sample
	Mix and incubate.	On ice for 5 minutes	On ice for 5 minutes
6	**Add cold 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, three more times (total of 4 x 3-minute separations)	Steps 6 and 7, three more times (total of 4 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

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 CD138 Positive Selection Kit Protocol

		EASYSEP™ MAGNETS	
		EasyEights™ (Catalog #18103)	
STEP	INSTRUCTIONS	5 mL tube	14 mL tube
1	Prepare sample at the indicated cell concentration within the volume range and keep cold until use.	1 x 10^8 cells/mL 0.5 - 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. Corning Catalog #352058)	14 mL (17 x 100 mm) polystyrene round-bottom tube (e.g. Corning Catalog #352057)
2	Add FcR blocker to sample.	50 µL/mL of sample	50 µL/mL of sample
3	Add Selection Cocktail to sample.	50 µL/mL of sample	50 µL/mL of sample
	Mix and incubate.	On ice for 5 minutes	On ice for 5 minutes
4	Vortex RapidSpheres™.	30 seconds	30 seconds
5	Add RapidSpheres™ to sample.	50 µL/mL of sample	50 µL/mL of sample
	Mix and incubate.	On ice for 5 minutes	On ice for 5 minutes
6	Add cold recommended medium to top up 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 10 minutes	RT for 10 minutes
7	Carefully pipette** (do not pour) 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 10-minute separations)	Steps 6 and 7, two more times (total of 3 x 10-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)

** Collect the entire supernatant, all at once, into a single pipette (e.g. for the EasyEights™ 5 mL tube use a 2 mL serological pipette and for the EasyEights™ 14 mL tube use a 10 mL serological pipette).

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™ Human PE Positive Selection Kit Protocol

STEP	INSTRUCTIONS	RoboSep™ (Catalog #20000 and #21000) 
1	Prepare sample at the indicated cell concentration within the volume range and keep cold until use.	1 x 10^8 cells/mL 0.5 - 8 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 CD138 Positive Selection 18957
3	Vortex RapidSpheres™.	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

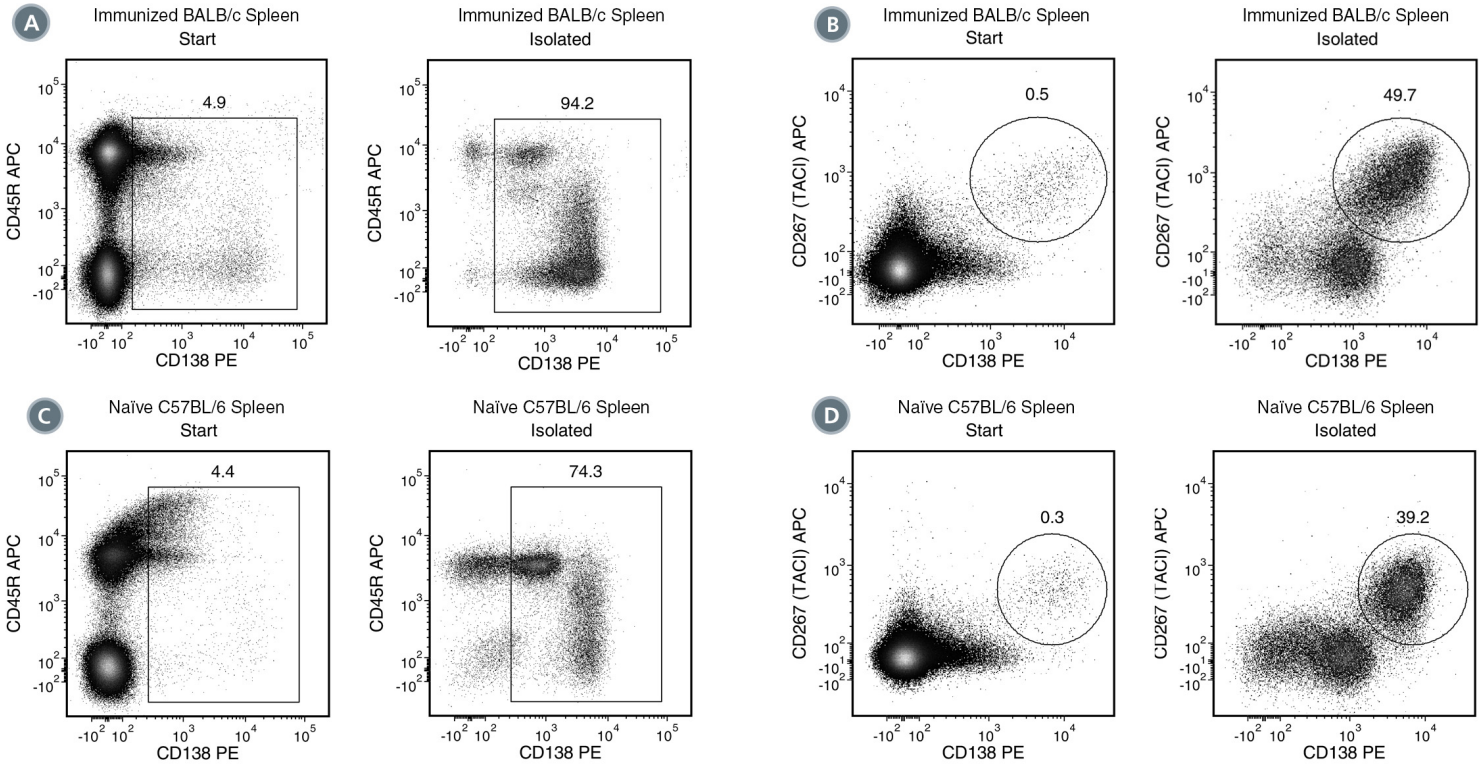
Notes and Tips

ASSESSING PURITY

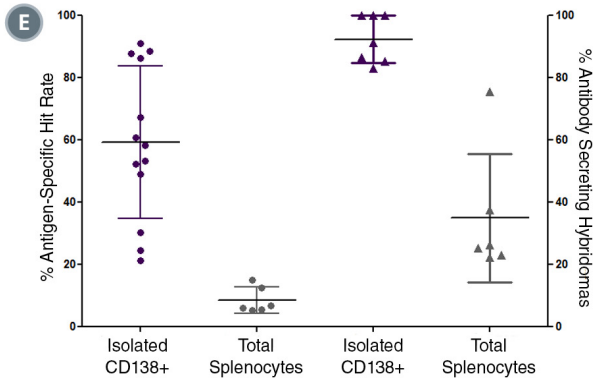
For purity assessment by flow cytometry use the following fluorochrome-conjugated antibody clones:

- Anti-Mouse CD138 (Syndecan-1) Antibody, Clone 281-2 (Catalog #60035; partially blocked), and
- Anti-Mouse CD45R Antibody, Clone RA3-6B2 (Catalog #60019), and
- Anti-Mouse CD267 (TACI) Antibody, Clone 8F10 (Catalog #60116)

Data



Starting with immunized BALB/c mouse splenocytes (A), the CD138+ cell content of the isolated fraction is typically 86.8 ± 6.8%, whereas the plasma cell (CD138+CD267 (TACI)+) content is typically 47.3 ± 1.7 % (B). Starting with naïve C57BL/6 mouse splenocytes (C), the CD138+ cell content of the isolated fraction is typically 64.0 ± 11.6%, whereas the plasma cell (CD138+CD267 (TACI)+) content is typically 29.4 ± 8.8 % (D).



(E) Isolated CD138+ cells or total splenocytes from immunized mice were fused with Sp2/0 mouse myeloma cells and plated in semi-solid ClonaCell™ medium (Catalog #03800). The % antigen-specific hit rate (circles) and % antibody-secreting hybridomas (triangles) were determined by ELISA. The % antigen-specific hit rates for CD138+ cells and total splenocytes were 57.2 ± 24.8% and 8.6 ± 4.2%, respectively. The % antibody-secreting hybridomas for CD138+ cells and total splenocytes were 92.3 ± 7.6% and 35.0 ± 20.6%, respectively.

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