EasySep™ Mouse B Cell Isolation Kit

Negative Selection

Catalog #19854

For processing 1 x 10^9 cells

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Document #29265 | Version 1_1_2

Description

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

- Fast and easy-to-use
- Up to 98% purity
- No column required
- Untouched, viable cells

This kit targets non-B cells for removal with biotinylated antibodies recognizing specific cell surface markers. Unwanted cells are labeled with biotinylated antibodies and streptavidin-coated 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 B Cell Isolation Cocktail	19854C	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.
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

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

SPLEEN

Disrupt spleen in PBS or Hanks' Balanced Salt Solution (HBSS) 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 recommended medium.

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

Recommended Medium

EasySep™ Buffer (Catalog #20144), RoboSep™ Buffer (Catalog #20104), or PBS (Catalog #37350) containing 2% 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 Tables 1 and 2 for detailed instructions regarding the EasySep™ procedure for each magnet.

Table 1. EasySep™ Mouse B 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 0.25 - 2 mL	1 x 10^8 cells/mL 0.5 - 8 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.	RT for 10 minutes	RT for 10 minutes
5	**Vortex RapidSpheres™.**	30 seconds	30 seconds
6	**Add RapidSpheres™ to sample.**	75 µL/mL of sample	75 µL/mL of sample
	Mix and incubate.	RT for 2.5 minutes	RT for 2.5 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 2.5 minutes	RT for 2.5 minutes
8	**Pick up the magnet, and in one continuous motion invert the magnet and tube,* pouring off the enriched cell suspension into a new tube.**	Isolated cells are now ready for use	Isolated cells are now 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 B Cell Isolation 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 0.05 - 0.2 mL	1 x 10^8 cells/mL 0.25 - 2 mL	1 x 10^8 cells/mL 0.5 - 8 mL
2	Add Rat Serum to sample.	50 µL/mL of sample	50 µL/mL of sample	50 µL/mL of sample
3	Add sample to required tube (or plate if using the EasyPlate™ EasySep™ Magnet).	Round-bottom, non-tissue culture-treated 96-well plate (e.g. Costar Catalog #3788)	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	50 µL/mL of sample
	Mix and incubate.	RT for 10 minutes	RT for 10 minutes	RT for 10 minutes
5	Vortex RapidSpheres™.	30 seconds	30 seconds	30 seconds
6	Add RapidSpheres™ to sample.	75 µL/mL of sample	75 µL/mL of sample	75 µL/mL of sample
	Mix and incubate.	RT for 2.5 minutes	RT for 2.5 minutes	RT for 2.5 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 0.25 mL	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 or plate (without lid) into the magnet and incubate.	RT for 2.5 minutes	RT for 5 minutes	RT for 5 minutes
8	Carefully pipette (do not pour) the enriched cell suspension** into a new tube or plate.	Isolated cells are now ready for use	Isolated cells are now ready for use	Isolated cells are now 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™ Mouse B Cell Isolation 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	**Select protocol.**	Mouse B Cell Isolation 19854
5	**Vortex RapidSpheres™.**	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. Remove the tube containing the isolated cells.**	Isolated cells are now ready for use

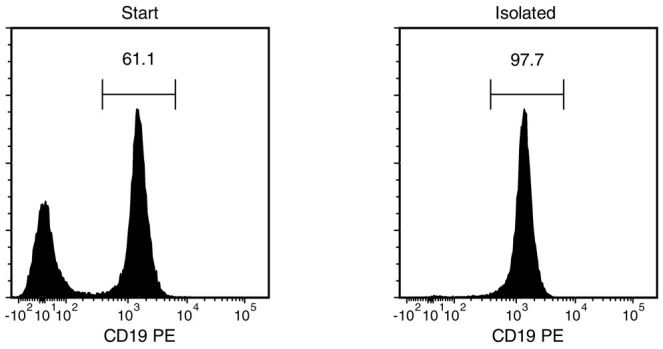
Notes and Tips

ASSESSING PURITY

For purity assessment of B cells by flow cytometry use fluorochrome-conjugated:

- Anti-Mouse CD19 Antibody, Clone 6D5 (Catalog #60006)

Data



Starting with mouse splenocytes, the B cell content (CD19+) of the isolated fraction is typically ranges from 94 - 98%. In the above example, the final purities of the start and isolated fractions are 61.1% and 97.7%, respectively.