



EasySep™ Mouse B Cell Enrichment Kit

Negative Selection

Catalog #19754

For processing 1 x 10⁹ cells



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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, easy-to-use and column-free
- Up to 96% purity
- 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 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 or cell-based assays.

Component Descriptions

COMPONENT NAME	COMPONENT #	QUANTITY	STORAGE	SHELF LIFE	FORMAT
EasySep™ Mouse B Cell Enrichment Cocktail	19754C.2	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™ Biotin Selection Cocktail	19153	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.
EasySep™ D Magnetic Particles	19250	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 TBS.
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; TBS - TRIS-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 10⁸ 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++ 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 B Cell Enrichment 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 Enrichment Cocktail to sample.	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	Add Selection Cocktail to sample.	100 µL/mL of sample	100 µL/mL of sample
	Mix and incubate.	2 - 8°C for 15 minutes	2 - 8°C for 15 minutes
6	Vortex Magnetic Particles.	30 seconds	30 seconds
7	Add Magnetic Particles to sample.	100 µL/mL of sample	100 µL/mL of sample
	Mix and incubate.	2 - 8°C for 5 minutes	2 - 8°C for 5 minutes
8	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	<ul style="list-style-type: none"> • 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
9	Pick up the magnet, and in one continuous motion invert the magnet and tube,* pouring the enriched cell suspension into a new 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 B Cell Enrichment Kit Protocol

STEP	INSTRUCTIONS	EASYSEP™ MAGNETS				
		EasyPlate™ (Catalog #18102)	EasyEights™ (Catalog #18103)	5 mL tube	14 mL tube	Easy 50 (Catalog #18002)
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	1 x 10 ⁸ cells/mL 1 - 40 mL	
2	Add Rat Serum to sample.	50 µL/mL of sample	50 µL/mL of sample	50 µL/mL of sample	50 µL/mL of sample	50 µL/mL of sample
3	Add sample to required tube (or plate when using the EasyPlate™ EasySep™ Magnet).	Round bottom, non-tissue culture-treated 96-well plate (e.g. Costar Catalog #3788 or Corning Catalog #351177)	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)	50 mL conical tube (e.g. Corning Catalog #352070)	
4	Add Enrichment Cocktail to sample.	50 µL/mL of sample	50 µL/mL of sample	50 µL/mL of sample	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	2 - 8°C for 15 minutes	2 - 8°C for 15 minutes	2 - 8°C for 15 minutes
5	Add Selection Cocktail to sample.	100 µL/mL of sample	100 µL/mL of sample	100 µL/mL of sample	100 µL/mL of sample	100 µL/mL of sample
	Mix and incubate.	2 - 8°C for 15 minutes	2 - 8°C for 15 minutes	2 - 8°C for 15 minutes	2 - 8°C for 15 minutes	2 - 8°C for 15 minutes
6	Vortex Magnetic Particles.	30 seconds	30 seconds	30 seconds	30 seconds	30 seconds
7	Add Magnetic Particles to sample.	150 µL/mL of sample	100 µL/mL of sample	100 µL/mL of sample	100 µL/mL of sample	100 µL/mL of sample
	Mix and incubate.	2 - 8°C for 5 minutes	2 - 8°C for 5 minutes	2 - 8°C for 5 minutes	2 - 8°C for 5 minutes	2 - 8°C for 10 minutes
8	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	<ul style="list-style-type: none"> Top up to 5 mL for samples < 4 mL Top up to 10 mL for samples ≥ 4 mL 	<ul style="list-style-type: none"> Top up to 25 mL for start samples ≤ 10 mL Top up to 50 mL for start samples > 10 mL 	
	Place the tube or plate (without lid) into the magnet and incubate.	RT for 10 minutes	RT for 5 minutes	RT for 5 minutes	RT for 10 minutes	
9	Carefully pipette (do not pour) the enriched cell suspension** into a new tube or plate.	Isolated cells are ready for use	Isolated cells are ready for use	Isolated cells are ready for use	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™ Mouse B Cell Enrichment 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 Negative Selection 19754
5	Vortex Magnetic Particles.	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

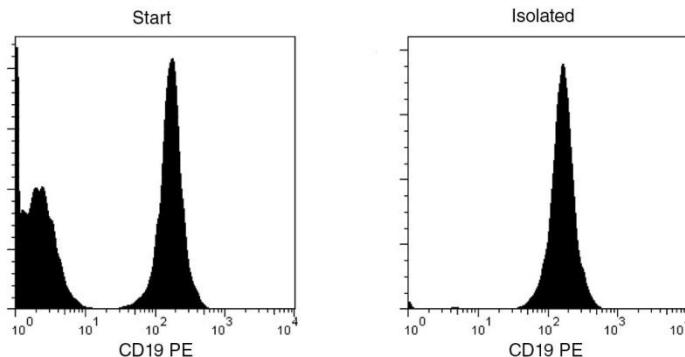
Notes and Tips

ASSESSING PURITY

For purity assessment of B cells by flow cytometry use the following fluorochrome-conjugated antibody clone:

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

Data



Starting with mouse splenocytes, the B cell content (CD19+) of the enriched fraction typically ranges from 91 - 96%. In the above example, the purities of the start and final isolated fractions are 50.3% and 96.4%, respectively.

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