EasySep™ Mouse CD19 Positive Selection Kit II

Positive Selection

Catalog #18954

For labeling up to 2 x 10e9 cells

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Description

Isolate highly purified CD19+ cells from mouse single-cell suspensions of splenocytes or other tissues by immunomagnetic positive selection.

- Fast and easy-to-use
- Up to 99% purity
- No columns required
- Isolated cells are not fluorochrome labeled

This kit targets CD19+ cells for positive selection with antibodies recognizing the CD19 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 cell-based experiments.

Component Descriptions

COMPONENT NAME	COMPONENT #	QUANTITY	STORAGE	SHELF LIFE	FORMAT
EasySep™ Mouse CD19 Positive Selection Kit II Component A	18954CA	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 with 0.1% BSA and 10% HPCD.
EasySep™ Mouse CD19 Positive Selection Kit II Component B	18954CB	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 with 0.1% BSA and 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.
RoboSep™ Empty Vial	27401	1	Not applicable	Not applicable	Not applicable

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

Components may be shipped at room temperature (15 - 25°C) and should be stored according to their storage conditions upon receipt.

Additional Reagent Stability Information

REAGENT NAME	STORAGE	SHELF LIFE
Selection Cocktail (combined Component A + Component B)	Store at 2 - 8°C. Do not freeze.	Stable for up to 4 weeks. Do not exceed expiry date (EXP) of individual components.

Sample Preparation

Disrupt spleen in 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 10e8 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 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 CD19 Positive Selection Kit II 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 10e8 cells/mL 0.25 - 2 mL	1 x 10e8 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	**Prepare Selection Cocktail in a tube. For each 1 mL of sample make 50 µL of cocktail (25 µl of Component A + 25 µL of Component B).**	Mix equal volumes of Component A and Component B. Prepared cocktail is stable at 2 - 8°C for up to 4 weeks.	Mix equal volumes of Component A and Component B. Prepared cocktail is stable at 2 - 8°C for up to 4 weeks.
	Incubate.	RT for 5 minutes	RT for 5 minutes
3	**Add Selection Cocktail to sample.**	50 µL/mL of sample	50 µL/mL of sample
	Mix and incubate.	RT for 3 minutes	RT for 3 minutes
4	**Vortex RapidSpheres™.**	30 seconds	30 seconds
5	**Add RapidSpheres™ to sample.**	75 µL/mL of sample	75 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 3 mL for samples < 2.5 mL • Top up to 10 mL for samples ≥ 2.5 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 (total of 2 x 3-minute separations)	Steps 6 and 7 (total of 2 x 3-minute separations)
9	**Resuspend cells in desired medium. Be sure to collect cells from the sides of the 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 CD19 Positive Selection Kit II 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.	1 x 10e8 cells/mL 0.5 - 2 mL	1 x 10e8 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	Prepare Selection Cocktail in a tube. For each 1 mL of sample make 50 µL of cocktail (25 µl of Component A + 25 µL of Component B).	Mix equal volumes of Component A and Component B. Prepared cocktail is stable at 2 - 8°C for up to 4 weeks.	Mix equal volumes of Component A and Component B. Prepared cocktail is stable at 2 - 8°C for up to 4 weeks.
	Incubate.	RT for 5 minutes	RT for 5 minutes
3	Add Selection Cocktail to sample.	50 µL/mL of sample	50 µL/mL of sample
	Mix and incubate.	RT for 3 minutes	RT for 3 minutes
4	Vortex RapidSpheres™.	30 seconds	30 seconds
5	Add RapidSpheres™ to sample.	75 µL/mL of sample	75 µL/mL of sample
	Mix and incubate.	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 2.5 mL	• Top up to 3 mL for samples < 2.5 mL • Top up to 10 mL for samples ≥ 2.5 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	Add 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 3 mL for samples < 2.5 mL • Top up to 10 mL for samples ≥ 2.5 mL
	Place the tube (without lid) into the magnet and incubate.	RT for 5 minutes	RT for 5 minutes
9	Carefully pipette** (do not pour) off the supernatant. Remove the tube from the magnet; this tube contains the isolated cells.	Discard supernatant	Discard supernatant
10	Resuspend cells in desired medium. Be sure to collect cells from the sides of the tube.	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 CD19 Positive Selection Kit Protocol

STEP	INSTRUCTIONS	RoboSep™ (Catalog #20000 and #21000) 
1	Prepare sample at the indicated cell concentration within the volume range.	1 x 10e8 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	Prepare Selection Cocktail in the RoboSep™ Empty Vial provided. See Table 4 for required volumes.	Mix equal volumes of Component A and Component B (see Table 4). Prepared cocktail is stable at 2 - 8°C for up to 4 weeks.
	Incubate.	RT for 5 minutes
3	Select protocol.	Mouse CD19 Positive Selection II 18954v2
4	Vortex RapidSpheres™.	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 now ready for use

RT - room temperature (15 - 25°C)

Table 4. RoboSep™ Selection Cocktail Preparation

START SAMPLE	COMPONENT A	COMPONENT B	SELECTION COCKTAIL TOTAL VOLUME
0.5 mL	62.5 µL	62.5 µL	125 µL
1 mL	75 µL	75 µL	150 µL
1.5 mL	87.5 µL	87.5 µL	175 µL
2 mL	100 µL	100 µL	200 µL
3 mL	125 µL	125 µL	250 µL
4 mL	150 µL	150 µL	300 µL
5 mL	175 µL	175 µL	350 µL
6 mL	200 µL	200 µL	400 µL
7 mL	225 µL	225 µL	450 µL
8 mL	250 µL	250 µL	500 µL

Note: RoboSep™ requires an extra 100 µL of the Selection Cocktail to run properly (compared to manual protocols).

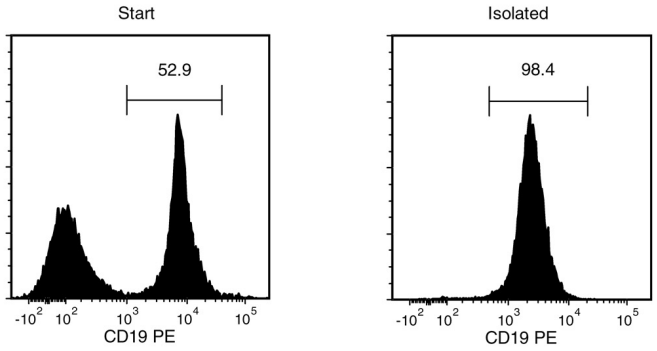
Notes and Tips

ASSESSING PURITY

The CD19 selection cocktail uses the anti-CD19 antibody clone 6D5. For purity assessment by flow cytometry use the following fluorochrome-conjugated antibody clone:

- anti-mouse CD19 antibody, clone 1D3 (partially blocked)

Data



Starting with mouse splenocytes, the CD19+ cell content of the isolated fraction is typically 98.1 ± 0.6% (mean ± SD using the purple EasySep™ Magnet).