EasySep™ Mouse Memory CD4+ T Cell Isolation Kit

For processing 1 x 10^9 cells

Catalog #19767
Catalog #19767RF RoboSep™

Negative Selection

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Description

Isolate untouched and highly purified memory CD4+ T cells (CD4+CD62L^{high}CD44$^{high/int}$ central memory and CD4+CD62L-CD44$^{high/int}$ effector memory) 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-memory CD4+ T 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 Memory CD4+ T Cell Isolation Cocktail	19767C	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	2 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.
EasySep™ Mouse Fcr Blocker	18730	1 x 0.2 mL	Store at 2 - 8°C. Do not freeze.	Stable until expiry date (EXP) on label.	A combination of monoclonal antibodies in PBS, 0.1% BSA, and < 0.1% sodium azide.

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

SPLEEN

Disrupt spleen in PBS or Hanks' Balanced Salt Solution (HBSS) containing 2% fetal bovine serum (FBS). 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.

Recommended Medium

EasySep™ Buffer (Catalog #20144), RoboSep™ Buffer (Catalog #20104), or PBS 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 Memory CD4+ T 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 0.5 - 2 mL	1 x 10^8 cells/mL 0.5 - 8 mL
2	Add Mouse FcR Blocker to sample.	20 µL/mL of sample	20 µL/mL of sample
3	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)
4	Add Isolation Cocktail to sample. NOTE: Do not vortex cocktail.	50 µL/mL of sample	50 µL/mL of sample
	Mix and incubate.	RT for 15 minutes	RT for 15 minutes
5	Vortex RapidSpheres™. NOTE: Particles should appear evenly dispersed.	30 seconds	30 seconds
6	Add RapidSpheres™ to sample.	125 µL/mL of sample	125 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 5 minutes	RT for 5 minutes
8	Pick up the magnet, and in one continuous motion invert the magnet and tube,* pouring the enriched cell suspension into a new tube.	Use a new 14 mL tube	• Use a new 14 mL tube for start samples < 4 mL • Use a new 50 mL tube for start samples ≥ 4 mL
9	Remove the tube from the magnet and add recommended medium to the indicated volume. Mix by gently pipetting up and down 5 - 6 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
10	Place the tube (without lid) into the magnet and incubate.	RT for 5 minutes	RT for 5 minutes
11	Pick up the magnet, and in one continuous motion invert the magnet and tube,* pouring the enriched cell suspension.	Combine with first poured-off fraction from step 8 Isolated cells are ready for use	Combine with first poured-off fraction from step 8 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.

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Table 2. EasySep™ Mouse Memory CD4+ T Cell Isolation 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.	1 x 10^8 cells/mL 0.25 - 2 mL	1 x 10^8 cells/mL 0.5 - 8 mL
2	Add Mouse FcR Blocker to sample.	20 µL/mL of sample	20 µL/mL of sample
3	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)
4	Add Isolation Cocktail to sample. NOTE: Do not vortex cocktail.	50 µL/mL of sample	50 µL/mL of sample
	Mix and incubate.	RT for 15 minutes	RT for 15 minutes
5	Vortex RapidSpheres™. NOTE: Particles should appear evenly dispersed.	30 seconds	30 seconds
6	Add RapidSpheres™ to sample.	125 µL/mL of sample	125 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 5 minutes	RT for 5 minutes
8	Carefully pipette** (do not pour) the enriched cell suspension into a new tube.	Use a new 14 mL tube	• Use a new 14 mL tube for start samples < 4 mL • Use a new 50 mL tube for start samples ≥ 4 mL
9	Remove the tube from the magnet and add recommended medium to the indicated volume. Mix by gently pipetting up and down 5 - 6 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
10	Place the tube (without lid) into the magnet and incubate.	RT for 5 minutes	RT for 5 minutes
11	Carefully pipette** (do not pour) the enriched cell suspension into a new tube.	Combine with first fraction from step 8 Isolated cells are ready for use	Combine with first fraction from step 8 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 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 Memory CD4+ T Cell Isolation 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 1 - 6.5 mL
2	**Add Mouse FcR Blocker to sample.**	20 µL/mL of sample
3	**Add sample to required tube.**	14 mL (17 x 95 mm) polystyrene round-bottom tube (e.g. Catalog #38008)
4	**Select protocol.**	Mouse Memory CD4+ T Cell Isolation 19767
5	**Vortex RapidSpheres™.** **NOTE: Particles should appear evenly dispersed.**	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 memory CD4+ T cells by flow cytometry, use the following fluorochrome-conjugated antibody clones:

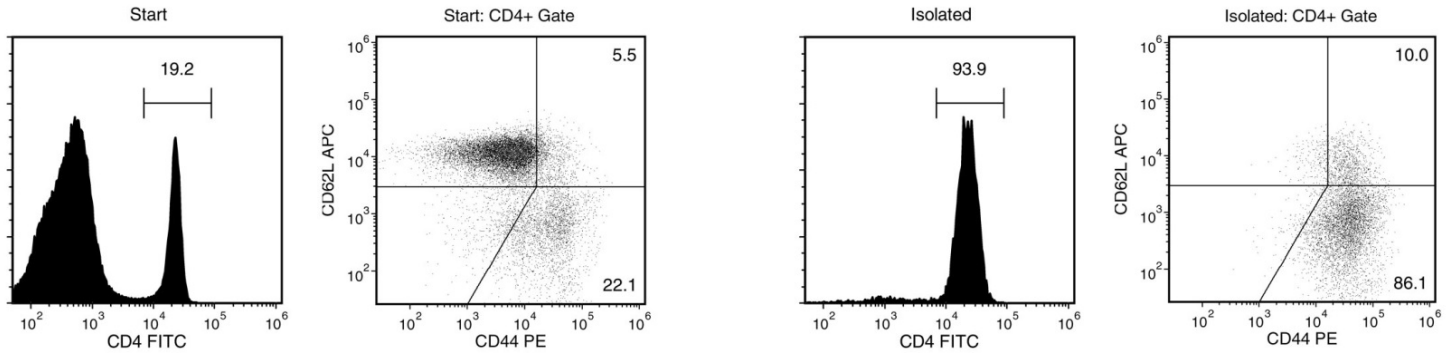
- Anti-Mouse CD4 Antibody, Clone RM4-5 (Catalog #60017), and
- Anti-Mouse CD44 Antibody, Clone IM7 (Catalog #60068), and
- Anti-Mouse CD62L (L-Selectin) Antibody, Clone MEL-14 (Catalog #60109)

Mouse central memory CD4+ T cells will be CD4+CD62L^{high}CD44$^{high/int}$, and effector memory CD4+ T cells will be CD4+CD62L-CD44$^{high/int}$.

INCUBATION TEMPERATURE

Protocols may be performed at 2 - 8°C without affecting the performance of the kit.

Data



Starting with mouse splenocytes, the memory CD4+ T cell content (CD4+CD62L^{high}CD44$^{high/int}$ central memory and CD4+CD62L-CD44$^{high/int}$ effector memory) of the isolated fraction typically ranges from 78 - 96%. In the above example, the purities of the start and final isolated fractions are 5.3% and 90.2%, respectively.