EasySep™ Mouse Pan-Naïve T Cell Isolation Kit

For processing 1 x 10^9 cells

Catalog #19848
#19848RF RoboSep™

Negative Selection

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Description

Isolate untouched and highly purified pan-naïve T cells (CD3+CD44-/^{low}CD62L^{high}) 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 97% purity
- Untouched, viable cells

This kit targets non-naïve 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, or cell-based assays.

Component Descriptions

COMPONENT NAME	COMPONENT #	QUANTITY	STORAGE	SHELF LIFE	FORMAT
EasySep™ Mouse T Cell Isolation Cocktail	19851C.1	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™ Mouse Memory T Cell Depletion Cocktail	18766C	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.
EasySep™ Mouse FcR Blocker	18720	1 x 0.1 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 Pan-Naïve 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 0.1 - 2 mL	1 x 10^8 cells/mL 0.25 - 8 mL
2	**Add FcR blocker to sample.**	10 µL/mL of sample	10 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 7.5 minutes	RT for 7.5 minutes
5	**Add Depletion Cocktail to sample.** **NOTE: Do not vortex cocktail.**	50 µL/mL of sample	50 µL/mL of sample
	Mix and incubate.	RT for 2.5 minutes	RT for 2.5 minutes
6	**Vortex RapidSpheres™.** **NOTE: Particles should appear evenly dispersed.**	30 seconds	30 seconds
7	**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
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	• 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
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 Pan-Naïve T Cell Isolation Kit Protocol

		EASYSEP™ MAGNETS		
STEP	INSTRUCTIONS	 EasyPlate™ (Catalog #18102)	 EasyEights™ (Catalog #18103) 5 mL tube	EasyEights™ (Catalog #18103) 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 0.2 - 2 mL	1 x 10^8 cells/mL 0.2 - 8 mL
2	Add FcR blocker to sample.	10 µL/mL of sample	10 µL/mL of sample	10 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. Catalog #38018)	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	50 µL/mL of sample
	Mix and incubate.	RT for 7.5 minutes	RT for 7.5 minutes	RT for 7.5 minutes
5	Add Depletion Cocktail to sample. NOTE: Do not vortex cocktail.	50 µL/mL of sample	50 µL/mL of sample	50 µL/mL of sample
	Mix and incubate.	RT for 2.5 minutes	RT for 2.5 minutes	RT for 2.5 minutes
6	Vortex RapidSpheres™. NOTE: Particles should appear evenly dispersed.	30 seconds	30 seconds	30 seconds
7	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
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	• 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 2.5 minutes	RT for 2.5 minutes
9	Carefully pipette** (do not pour) the enriched cell suspension into a new tube or plate.	Isolated cells are ready for use	Use a new 5 mL tube	Use a new 14 mL tube
10	Remove the tube from the magnet and place the new tube (without lid) into the magnet and incubate for a second separation.	---	RT for 2.5 minutes	RT for 2.5 minutes
11	Carefully pipette** (do not pour) 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)

** 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 Pan-Naïve 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 - 8 mL NOTE: If starting with fewer than 1 x 10^8 cells, resuspend in 1 mL.
2	Add FcR blocker to sample.	10 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 Pan-Naïve T Cell Isolation 19848 (19851/18766)
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

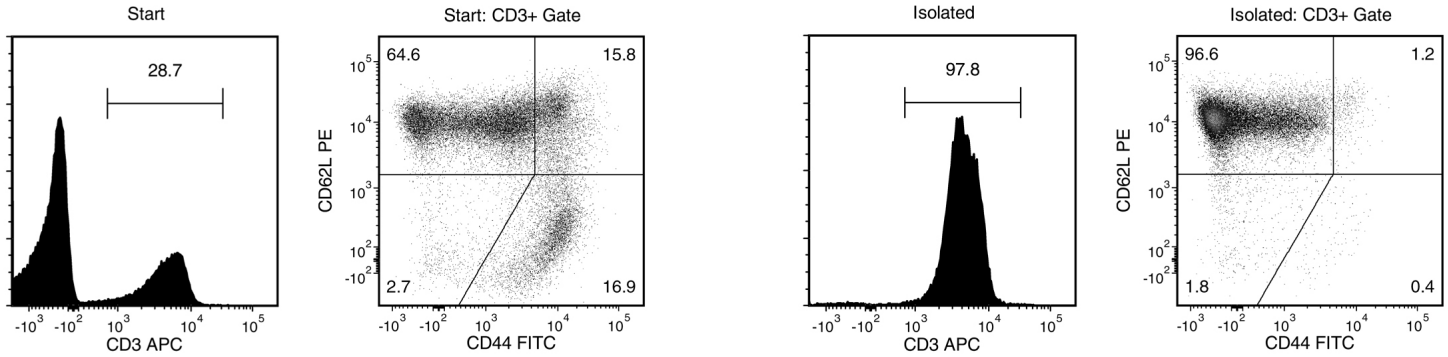
ASSESSING PURITY

For purity assessment of pan-naïve T cells (CD3+CD44-/^{low}CD62L^{high}) by flow cytometry, use the following fluorochrome-conjugated antibody clones:

- Anti-Mouse CD3e Antibody, Clone 145-2C11 (Catalog #60015), and
- Anti-mouse CD44 antibody, clone 5035-41.1D, and
- Anti-Mouse CD62L (L-Selectin) Antibody, Clone MEL-14 (Catalog #60109)

The anti-mouse CD44 antibody, clone 5035-41.1D only recognizes the Ly-24.2 isoform, which is expressed by C57BL/6, C57BL/10, C57/L, C58A, AKR, 129, SJL, NZB, C3H, CE, and CBA/H mouse strains.

Data



Starting with mouse splenocytes from an uninfected mouse, the pan-naïve T cell content (CD3+CD44-/^{low}CD62L^{high}) of the isolated fraction typically ranges from 90 - 97%. In the above example, the purities of the start and final isolated fractions are 18.5% and 94.5%, respectively.