



## EasySep™ Mouse Naïve CD4+ T Cell Isolation Kit

Negative Selection  
Catalog #19765

For processing 1 x 10<sup>9</sup> cells



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## Description

Isolate untouched and highly purified naïve CD4+ T cells (CD4+CD44<sup>low</sup>CD62L<sup>high</sup>) 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 95% purity
- Untouched, viable cells

This kit targets non-naïve CD4+ T 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 CD4+ T Cell Isolation Cocktail	19852C.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.
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 containing 2% fetal bovine serum (FBS). Remove aggregates and debris by passing cell suspension through a 70 µm mesh nylon strainer (e.g. Catalog #27260). Centrifuge at 300 x g for 10 minutes and resuspend at 1 x 10<sup>8</sup> nucleated cells/mL in recommended medium.

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



## Recommended Medium

RoboSep™ Buffer (Catalog #20104), or PBS containing 2% FBS. Medium should be free of Ca<sup>++</sup>, Mg<sup>++</sup>, 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 Naïve 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 <sup>8</sup> cells/mL 0.1 - 2 mL	1 x 10 <sup>8</sup> cells/mL 0.25 - 8 mL
	Add Rat Serum to sample.	50 µL/mL of sample	50 µL/mL of sample
2	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)
3	Add Isolation Cocktail to sample.	50 µL/mL of sample	50 µL/mL of sample
	Mix and incubate.	RT for 7.5 minutes	RT for 7.5 minutes
4	Add Depletion Cocktail to sample.	50 µL/mL of sample	50 µL/mL of sample
	Mix and incubate.	RT for 2.5 minutes	RT for 2.5 minutes
5	Vortex RapidSpheres™. NOTE: Particles should appear evenly dispersed.	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	<ul style="list-style-type: none"> <li>• Top up to 5 mL for samples &lt; 4 mL</li> <li>• Top up to 10 mL for samples ≥ 4 mL</li> </ul>
	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 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 Naïve CD4+ T Cell Isolation Kit Protocol**

		EASYSEP™ MAGNETS	
STEP	INSTRUCTIONS	EasyEights™ (Catalog #18103)	
		5 mL tube	14 mL tube
1	Prepare sample at the indicated cell concentration within the volume range.	1 x 10 <sup>8</sup> cells/mL 0.1 - 2 mL	1 x 10 <sup>8</sup> cells/mL 0.25 - 8 mL
	Add Rat Serum to sample.	50 µL/mL of sample	50 µL/mL of sample
2	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)
3	Add Isolation Cocktail to sample.	50 µL/mL of sample	50 µL/mL of sample
	Mix and incubate.	RT for 7.5 minutes	RT for 7.5 minutes
4	Add Depletion Cocktail to sample.	50 µL/mL of sample	50 µL/mL of sample
	Mix and incubate.	RT for 2.5 minutes	RT for 2.5 minutes
5	Vortex RapidSpheres™. NOTE: Particles should appear evenly dispersed.	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 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"> <li>• Top up to 5 mL for samples &lt; 4 mL</li> <li>• Top up to 10 mL for samples ≥ 4 mL</li> </ul>
	Place the tube (without lid) into the magnet and incubate.	RT for 10 minutes	RT for 10 minutes
8	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 Naïve CD4+ T 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 <sup>8</sup> cells/mL 1 - 8 mL
	Add Rat Serum to sample.	50 µL/mL of sample
2	Add sample to required tube.	14 mL (17 x 95 mm) polystyrene round-bottom tube (e.g. #38008)
3	Select protocol.	Mouse Naïve CD4+ T Cell Isolation 19765v2 (19852_18766)
4	Vortex RapidSpheres™. NOTE: Particles should appear evenly dispersed.	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.	Isolated cells are ready for use

## Notes and Tips

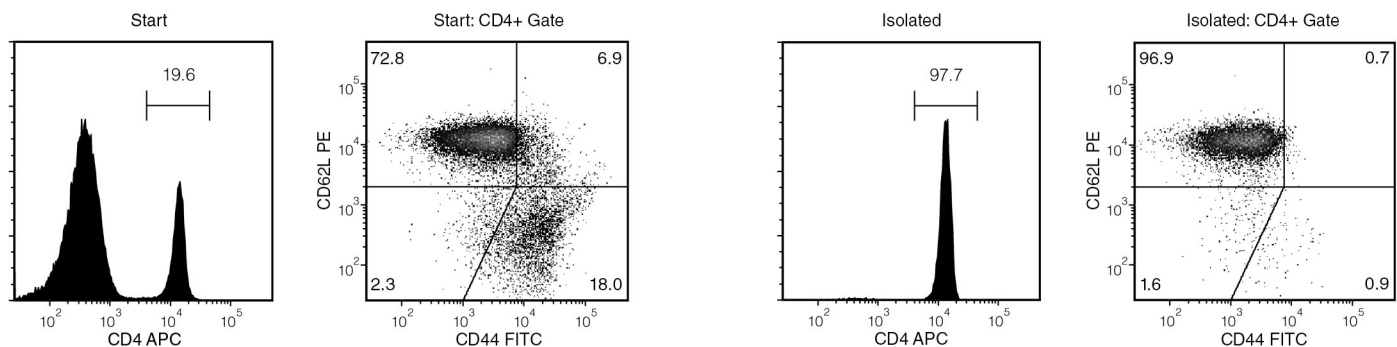
### ASSESSING PURITY

For purity assessment of naïve CD4+ T cells (CD4+CD44<sup>low</sup>CD62L<sup>high</sup>) 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 5035-41.1D, and
- Anti-Mouse CD62L (L-Selectin) Antibody, Clone MEL-14 (Catalog #60109)

The anti-mouse CD44 (Ly-24) clone 5035-41.1D is not blocked by the anti-CD44 clone used in EasySep™ Mouse Naïve CD4+ T Cell Isolation Cocktail. The 5035-41.1D clone 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. The anti-mouse/human CD44 clone IM7 is not recommended for assessing purity, as it is blocked by the anti-mouse CD44 antibody clone used in EasySep™ Mouse Naïve CD4+ T Cell Isolation Cocktail.

## Data



Starting with mouse splenocytes from an uninfected mouse, the naïve CD4+ T cell content (CD4+CD44<sup>low</sup>CD62L<sup>high</sup>) of the isolated fraction typically ranges from 87.6 - 95.6%. In the above example, the purities of the start and final isolated fractions are 14.3% and 94.7%, respectively.

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