



## EasySep™ Mouse CD4+ T Cell Enrichment Kit

Negative Selection

Catalog #19752

For labeling up to 1 x 10<sup>9</sup> cells



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## Description

Isolate untouched and highly purified CD4+ T 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 95% purity
- Untouched, viable cells

This kit targets non-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 Enrichment Cocktail	19752C.3	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 2	19653	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™ D2 Magnetic Particles	19650	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

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 10<sup>8</sup> 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<sup>++</sup> and Mg<sup>++</sup>; Catalog #37250) can be used in place of PBS. Medium should be free of Ca<sup>++</sup> and Mg<sup>++</sup>.

## 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 CD4+ T Cell Enrichment Kit Protocol**

		EASYSEP™ MAGNETS	
STEP	INSTRUCTIONS	 <b>EasySep™</b> (Catalog #18000)	<b>“The Big Easy”</b> (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
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"> <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 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 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 CD4+ T Cell Enrichment Kit Protocol**

		EASYSEPT™ MAGNETS		
STEP	INSTRUCTIONS	 <b>EasyPlate™</b> (Catalog #18102)	<b>EasyEights™ (Catalog #18103)</b>	
			 <b>5 mL tube</b>	 <b>14 mL tube</b>
1	Prepare sample at the indicated cell concentration within the volume range.	1 x 10e8 cells/mL 0.05 - 0.2 mL	1 x 10e8 cells/mL 0.25 - 2 mL	1 x 10e8 cells/mL 0.5 - 8 mL
2	Add Rat Serum to 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)
4	Add Enrichment Cocktail to 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
5	Add Selection Cocktail to 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
6	Vortex Magnetic Particles.	30 seconds	30 seconds	30 seconds
7	Add Magnetic Particles to 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
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"> <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 or plate (without lid) into the magnet and incubate.	RT for 10 minutes	RT for 5 minutes	RT for 5 minutes
9	Carefully pipette (do not pour) the enriched cell suspension** into a new tube or plate.	Isolated cells are now ready for use	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 CD4+ T 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 10e8 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 CD4+T Cell Negative Selection 19752 (D particles)	
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 now ready for use	

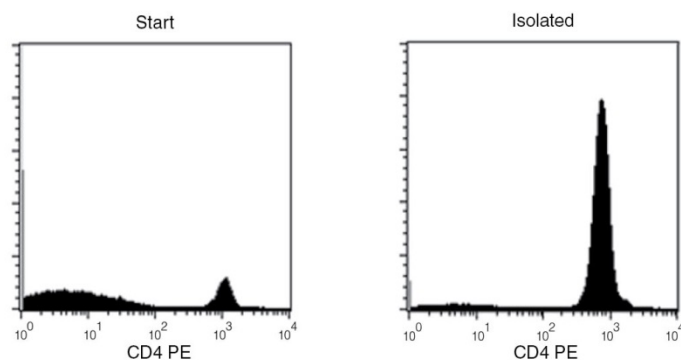
## Notes and Tips

### ASSESSING PURITY

For purity assessment of CD4+ T cells by flow cytometry use fluorochrome-conjugated:

- Anti-Mouse CD4 Antibody, Clone RM4-5 (Catalog #60017), or
- Anti-Mouse CD4 Antibody, Clone RM4-4 (Catalog #60029)

## Data



Starting with mouse splenocytes, the CD4+ cell content of the enriched fraction typically ranges from 84 - 95%. In the above example, the final purities of the start and isolated fractions are 22% and 94%, respectively.

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