EasySep™ Mouse CD4+CD62L+ T Cell Isolation Kit

For processing 1 x 10^9 cells

Catalog #18765
#18765RF RoboSep™

Positive Selection

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Description

Isolate highly purified naïve CD4+ T cells (CD4+CD62L+) from mouse splenocytes or other single-cell suspensions using a simple, two-step procedure. When using single-cell suspensions from other tissue types, this kit may require optimization.

- Fast and easy-to-use
- Up to 97% purity
- No columns required

First, naïve CD4+ T cells are pre-enriched using EasySep™ Mouse Naïve CD4+ T Cell Pre-Enrichment Cocktail (18765C) with antibodies recognizing specific cell surface markers. Then, cells pre-labeled with CD62L PE are selected using EasySep™ PE Selection Cocktail (18151). These EasySep™ cocktails label cells with antibodies that link to magnetic particles. The cells are separated without columns using an EasySep™ magnet. Isolated cells are immediately available for downstream applications, such as flow cytometry, cell culture, or DNA/RNA extraction.

Component Descriptions

COMPONENT NAME	COMPONENT #	QUANTITY	STORAGE	SHELF LIFE	FORMAT
EasySep™ Mouse Naïve CD4+ T Cell Pre-Enrichment Cocktail	18765C	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™ PE Selection Cocktail	18151	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™ 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™ 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.
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

For automated and standardized tissue processing, see STEMprep™ Tissue Dissociator (Catalog #100-2112) at www.stemcell.com/stemprep. For manual processing, follow the steps below.

SPLEEN

Disrupt spleen in recommended medium. 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 10^8 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% fetal bovine serum (FBS) with 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 Table 1 for detailed instructions regarding the EasySep™ procedure for each magnet.

Table 1. EasySep™ Mouse CD4+CD62L+ 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 - 8.5 mL
2	Add Mouse 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 Pre-Enrichment Cocktail to sample. NOTE: Do not vortex cocktail.	50 µL/mL of sample	50 µL/mL of sample
	Mix and incubate.	RT for 10 minutes	RT for 10 minutes
5	Vortex Streptavidin RapidSpheres™. NOTE: Particles should appear evenly dispersed.	30 seconds	30 seconds
6	Add Streptavidin RapidSpheres™ to sample.	75 µL/mL of sample NOTE: Two different particles are provided in this kit. Ensure that Streptavidin RapidSpheres™ 50001 are used in this step.	75 µL/mL of sample NOTE: Two different particles are provided in this kit. Ensure that Streptavidin RapidSpheres™ 50001 are used in this step.
	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 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 pre-enriched cell suspension into a new tube.	Use a new 5 mL tube	Use a new 14 mL tube
9	Centrifuge pre-enriched cells.	200 x g for 10 minutes at RT	200 x g for 10 minutes at RT
	Discard the supernatant and resuspend cell pellet at the indicated volume.§	• Resuspend in 0.1 mL for samples with start volume ≤ 0.3 mL • Resuspend in 0.25 mL for samples with start volume > 0.3 mL	• Resuspend in 0.5 mL for samples with start volume ≤ 2 mL • Resuspend in 2 mL for samples with start volume > 2 mL
Continue on to next page.		Continue on to next page.	Continue on to next page.

		EASYSEP™ MAGNETS	
STEP	INSTRUCTIONS (continued)	 **EasySep™ (Catalog #18000)**	**"The Big Easy" (Catalog #18001)** 
10	**Add PE Selection Cocktail to sample.** NOTE: Do not vortex cocktail.	40 µL/mL of sample	40 µL/mL of sample
	Mix and incubate.	RT for 5 minutes	RT for 5 minutes
11	**Vortex Dextran RapidSpheres™.** NOTE: Particles should appear evenly dispersed.	30 seconds	30 seconds
12	**Add Dextran RapidSpheres™ to sample.**	25 µL/mL of sample NOTE: Two different particles are provided in this kit. Ensure that Dextran RapidSpheres™ 50100 are used in this step.	25 µL/mL of sample NOTE: Two different particles are provided in this kit. Ensure that Dextran RapidSpheres™ 50100 are used in this step.
	Mix and incubate.	RT for 5 minutes	RT for 5 minutes
13	**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
14	**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
15	**Repeat steps as indicated.**	Steps 13 and 14 (total of 2 x 5-minute separations)	Steps 13 and 14 (total of 2 x 5-minute separations)
16	**Resuspend cells in desired medium. Be sure to collect cells from the sides of the 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.
§ The resuspended sample volume should be used to determine the volume of reagents added in subsequent steps.

Directions for Use – Fully Automated RoboSep™ Protocol

See page 1 for Sample Preparation and Recommended Medium. Refer to Table 2 for detailed instructions regarding the RoboSep™ procedure.

Table 2. RoboSep™ Mouse CD4+CD62L+ 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 0.5 - 8.5 mL
2	**Add Mouse 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 Naïve CD4+ T Cell Pre-Enrichment 18765
5	**Vortex Streptavidin RapidSpheres™.** **NOTE: Particles should appear evenly dispersed.**	30 seconds
6	**Load the carousel.**	Follow on-screen prompts NOTE: Two different particles are provided in this kit. Ensure that Streptavidin RapidSpheres™ 50001 are used in this step.
	Start the protocol.	Press the green “Run” button
7	**Unload the carousel when the run is complete.**	Remove the tube containing the pre-enriched cells
8	**Centrifuge the pre-enriched cells.**	200 x g for 10 minutes at RT
	Discard the supernatant and resuspend cell pellet at the indicated volume.	• Resuspend in 0.5 mL for samples with start volume ≤ 2 mL • Resuspend in 2 mL for samples with start volume > 2 mL
9	**Add sample to a new tube.**	14 mL (17 x 95 mm) polystyrene round-bottom tube (e.g. Catalog #38008)
10	**Select protocol.**	Mouse CD4+CD62L+ T Cell Positive Selection 18765 (18151)
11	**Vortex Dextran RapidSpheres™.** **NOTE: Particles should appear evenly dispersed.**	30 seconds
12	**Load the carousel.**	Follow on-screen prompts NOTE: Two different particles are provided in this kit. Ensure that Dextran RapidSpheres™ 50100 are used in this step.
	Start the protocol.	Press the green “Run” button
13	**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 ready for use

RT - room temperature (15 - 25°C)

Notes and Tips

ASSESSING PURITY

For purity assessment of naïve CD4+ T cells (CD4+CD44^{low} CD62L^{high}) 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)

NOTE: The positively selected CD62L+ cells have already been PE-labeled, so their purity can be assessed directly by flow cytometry.

Data

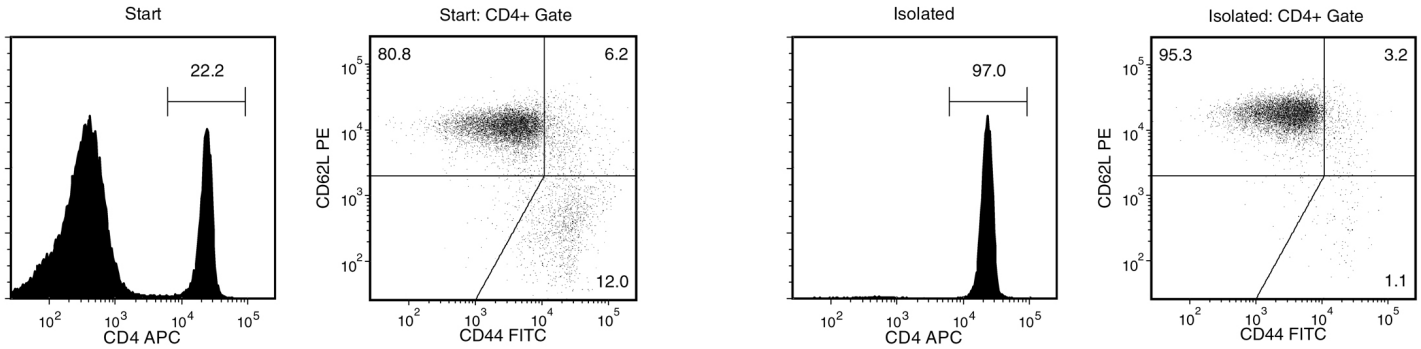

Figure 1. Isolation of Naïve CD4+ T Cells from Mouse Splenocytes

Starting with mouse splenocytes, the naïve CD4+ T cell content (CD4+CD44^{low} CD62L^{high}) of the final isolated fraction typically ranges from 91.7 - 96.7%. In the above example, the purities of the start and final isolated fractions are 17.9% and 92.4%, respectively.