



**EasySep™ Mouse
CD4+CD62L+ T Cell
Isolation Kit**

Positive Selection
Catalog #18765

For processing 1x 10⁹ cells



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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 the EasySep™ Mouse Naive CD4+ T Cell Pre-Enrichment Cocktail (18765C) with antibodies recognizing specific cell surface markers. Then, cells pre-labeled with CD62L PE are selected using the EasySep™ PE Selection Cocktail (18151). The 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 Naive 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.
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 recommended medium. 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 10⁸ 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. Hanks' Balanced Salt Solution (HBSS; 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 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 Pre-Enrichment Cocktail to sample.	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™.	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 Streptavidin RapidSpheres™ 50001 are used in this step.	75 µL/mL of sample NOTE: Two different particles are provided in this kit. Ensure 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	<ul style="list-style-type: none"> • 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 off the pre-enriched cell suspension into a new tube.	Use a new 5 mL tube	Use a new 14 mL tube
9	Wash the cells by topping up with recommended medium and centrifuge.	200 x g for 10 minutes at RT	200 x g for 10 minutes at RT
	Carefully remove supernatant and resuspend cell pellet in recommended medium to the indicated volume.	<ul style="list-style-type: none"> • Top up to 0.1 mL for start samples ≤ 0.3 mL • Top up to 0.25 mL for start samples > 0.3 mL 	<ul style="list-style-type: none"> • Top up to 0.5 mL for start samples ≤ 2 mL • Top up to 2 mL for start samples > 2 mL
Continue on to CD4+CD62L+ T Cell Selection Protocol.		Continue to next page	Continue to next page

		EASYSEP™ MAGNETS	
STEP	INSTRUCTIONS (continued)	 EasySep™ (Catalog #18000)	 “The Big Easy” (Catalog #18001)
10	Add PE Selection Cocktail to sample.	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™.	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 Dextran RapidSpheres™ 50100 are used in this step.	25 µL/mL of sample NOTE: Two different particles are provided in this kit. Ensure 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	<ul style="list-style-type: none"> • 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 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.

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+CD62L+ 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 ⁸ cells/mL 0.5 - 8.5 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 Naïve CD4+ T Cell Pre-Enrichment 18765	
5	Vortex Streptavidin RapidSpheres™.	30 seconds	
6	Load the carousel.	Follow on-screen prompts NOTE: Two different particles are provided in this kit. Ensure 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	Transfer sample to a new tube.	14 mL (17 x 100 mm) polystyrene round-bottom tube (e.g. Corning Catalog #352057)	
9	Wash the cells by topping up with recommended medium and centrifuge.	200 x g for 10 minutes at RT	
10	Carefully remove supernatant and resuspend cell pellet in recommended medium to the indicated volume.	<ul style="list-style-type: none"> • Top up to 0.5 mL for start samples ≤ 2 mL • Top up to 2 mL for start samples > 2 mL 	
Continue on to CD4+CD62L+ T Cell Selection Protocol.		---	
11	Vortex Dextran RapidSpheres™.	30 seconds	
12	Select protocol.	Mouse CD4+CD62L+ T Cell Positive Selection 18765 (18151)	
13	Load the carousel.	Follow on-screen prompts NOTE: Two different particles are provided in this kit. Ensure Dextran RapidSpheres™ 50100 are used in this step.	
	Start the protocol.	Press the green “Run” button	
14	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

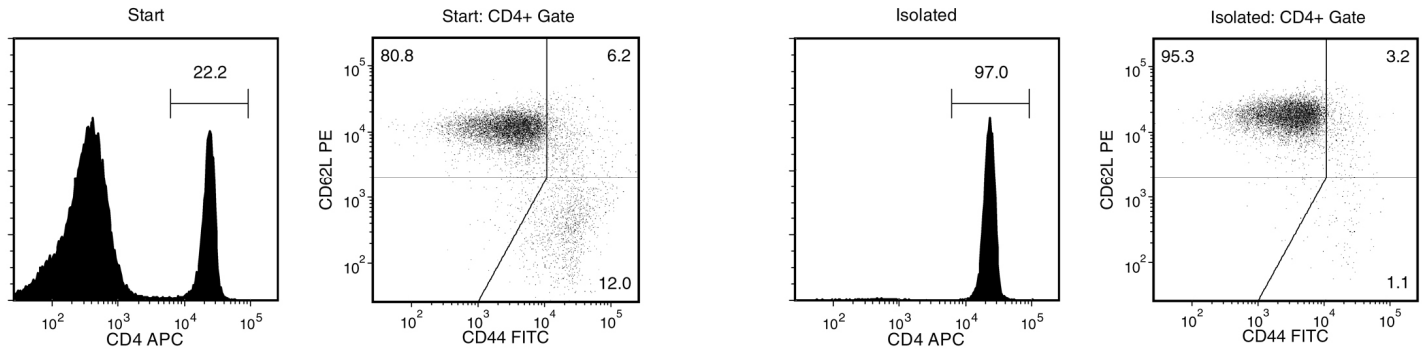
ASSESSING PURITY

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



Starting with a single-cell suspension of mouse splenocytes, the naïve CD4+ T cell (CD4+CD44^{low}CD62L^{high}) content 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.

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