EasySep™ Mouse CD4+CD25+ Regulatory T Cell Isolation Kit II

For processing 1 x 10^9 cells

Catalog #18783
#18783RF RoboSep™

Positive Selection

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Description

Isolate CD4+CD25+ cells 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, easy-to-use, and column-free
- Up to 93% purity

First, CD4+ T cells are pre-enriched using EasySep™ Mouse CD4+ T Cell Isolation Cocktail (19852C.1) with antibodies recognizing specific cell surface markers. Then, CD25+ are selected using EasySep™ Mouse CD25 Regulatory T Cell Positive Selection Cocktail (18782C), which contains antibodies recognizing CD25. 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 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 CD25 Regulatory T Cell Positive Selection Cocktail	18782C	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, and < 0.1% sodium azide.
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	18731	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, 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 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 10^8 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. Medium should be free of Ca++ and Mg++.

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+CD25+ Regulatory T Cell Isolation Kit II 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 0.5 - 2 mL	1 x 10^8 cells/mL 1 - 8 mL
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 CD4+ T Cell Isolation 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
4	**Vortex Streptavidin RapidSpheres™.** **NOTE: Particles should appear evenly dispersed.**	30 seconds	30 seconds
5	**Add Streptavidin RapidSpheres™ to sample.**	75 µL/mL of sample NOTE: Two different particles are provided in this kit. Ensure that Streptavidin RapidSpheres™ are used in this step.	75 µL/mL of sample NOTE: Two different particles are provided in this kit. Ensure that Streptavidin RapidSpheres™ are used in this step.
	Mix and incubate.	RT for 2.5 minutes	RT for 2.5 minutes
6	**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
7	**Pick up the magnet, and in one continuous motion invert the magnet and tube,* pouring the enriched cell suspension into a new tube.**	Use a new 5 mL tube	Use a new 14 mL tube
8	**Remove the tube from the magnet and place the new 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.**	Use a new 5 mL tube	Use a new 14 mL tube
10	**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 0.5 mL	Resuspend in 1 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)** 
11	**Add FcR Blocker to sample.**	25 µL	50 µL
	Mix and incubate.	RT for 5 minutes	RT for 5 minutes
12	**Add CD25 Regulatory T Cell Positive Selection Cocktail to sample.** **NOTE: Do not vortex cocktail.**	25 µL	50 µL
	Mix and incubate.	RT for 10 minutes	RT for 10 minutes
13	**Add PE Selection Cocktail to sample.** **NOTE: Do not vortex cocktail.**	10 µL	20 µL
	Mix and incubate.	RT for 5 minutes	RT for 5 minutes
14	**Vortex Dextran RapidSpheres™.** **NOTE: Particles should appear evenly dispersed.**	30 seconds	30 seconds
15	**Add Dextran RapidSpheres™ to sample.**	30 µL NOTE: Two different particles are provided in this kit. Ensure that Dextran RapidSpheres™ are used in this step.	60 µL NOTE: Two different particles are provided in this kit. Ensure that Dextran RapidSpheres™ are used in this step.
	Mix and incubate.	RT for 5 minutes	RT for 5 minutes
16	**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
17	**Pick up the magnet, and in one continuous motion invert the magnet and tube,* pouring off the supernatant.**	Discard supernatant	Discard supernatant
18	**Repeat steps as indicated.**	Steps 16 and 17, three more times (total of 4 x 5-minute separations)	Steps 16 and 17, three more times (total of 4 x 5-minute separations)
19	**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.

Table 2. EasySep™ Mouse CD4+CD25+ Regulatory T Cell Isolation Kit II 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 10^8 cells/mL 1 - 1.5 mL	1 x 10^8 cells/mL 1.5 - 8 mL
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 CD4+ T Cell Isolation 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
4	Vortex Streptavidin RapidSpheres™. NOTE: Particles should appear evenly dispersed.	30 seconds	30 seconds
5	Add Streptavidin RapidSpheres™ to sample.	75 µL/mL of sample NOTE: Two different particles are provided in this kit. Ensure that Streptavidin RapidSpheres™ are used in this step.	75 µL/mL of sample NOTE: Two different particles are provided in this kit. Ensure that Streptavidin RapidSpheres™ are used in this step.
	Mix and incubate.	RT for 2.5 minutes	RT for 2.5 minutes
6	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	• 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
7	Carefully pipette** (do not pour) the enriched cell suspension into a new tube.	Use a new 5 mL tube	Use a new 14 mL tube
8	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 0.5 mL	Resuspend in 1 mL
9	Add FcR Blocker to sample.	25 µL	50 µL
	Mix and incubate.	RT for 5 minutes	RT for 5 minutes
10	Add CD25 Regulatory T Cell Positive Selection Cocktail to sample. NOTE: Do not vortex cocktail.	25 µL	50 µL
	Mix and incubate.	RT for 10 minutes	RT for 10 minutes
11	Add PE Selection Cocktail to sample. NOTE: Do not vortex cocktail.	10 µL	20 µL
	Mix and incubate.	RT for 5 minutes	RT for 5 minutes
Continue on to next page.		Continue on to next page.	Continue on to next page.

STEP	INSTRUCTIONS	EasyEights™ (Catalog #18103)	
		5 mL tube	14 mL tube
12	Vortex Dextran RapidSpheres™. NOTE: Particles should appear evenly dispersed.	30 seconds	30 seconds
13	Add Dextran RapidSpheres™ to sample.	30 µL NOTE: Two different particles are provided in this kit. Ensure that Dextran RapidSpheres™ are used in this step.	60 µL NOTE: Two different particles are provided in this kit. Ensure that Dextran RapidSpheres™ are used in this step.
	Mix and incubate.	RT for 5 minutes	RT for 5 minutes
14	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	• 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 15 minutes	RT for 15 minutes
15	Carefully pipette** (do not pour) off the supernatant. Remove the tube, containing the isolated cells, from the magnet.	Discard supernatant	Discard supernatant
16	Repeat steps as indicated.	Steps 14 and 15, two more times (total of 3 x 15-minute separations)	Steps 14 and 15, two more times (total of 3 x 15-minute separations)
17	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)
** 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 CD4+CD25+ Regulatory T Cell Isolation Kit II 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
2	Add sample to required tube.	14 mL (17 x 95 mm) polystyrene round-bottom tube (e.g. Catalog #38008)
3	Select protocol.	Mouse CD4+ T Cell Pre-Enrichment 18783 (19852)
4	Vortex Streptavidin RapidSpheres™. NOTE: Particles should appear evenly dispersed.	30 seconds
5	Load the carousel.	Follow on-screen prompts NOTE: Two different particles are provided in this kit. Ensure that Streptavidin RapidSpheres™ are used in this step.
	Start the protocol.	Press the green “Run” button
6	Unload the carousel when the run is complete.	Remove the tube containing the isolated cells.
7	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 1 mL
8	Add sample to a new tube.	14 mL (17 x 95 mm) polystyrene round-bottom tube (e.g. Catalog #38008)
9	Add FcR blocker to sample.	50 µL
	Mix and incubate.	RT for 5 minutes
10	Select protocol.	Mouse CD25 Regulatory T Cell Positive Selection 18783 (18782)
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™ 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 CD4+CD25+ cells by flow cytometry, use the following fluorochrome-conjugated antibody clones:

- Anti-Mouse CD4 Antibody, Clone RM4-4 (Catalog #60029), or
- Anti-Mouse CD4 Antibody, Clone RM4-5 (Catalog #60017), and
- Anti-mouse FOXP3 antibody, clone FJK-16s (optional)

NOTE: The positively selected CD25+ cells have already been PE-labeled, so their purity can be assessed directly by flow cytometry.

Data

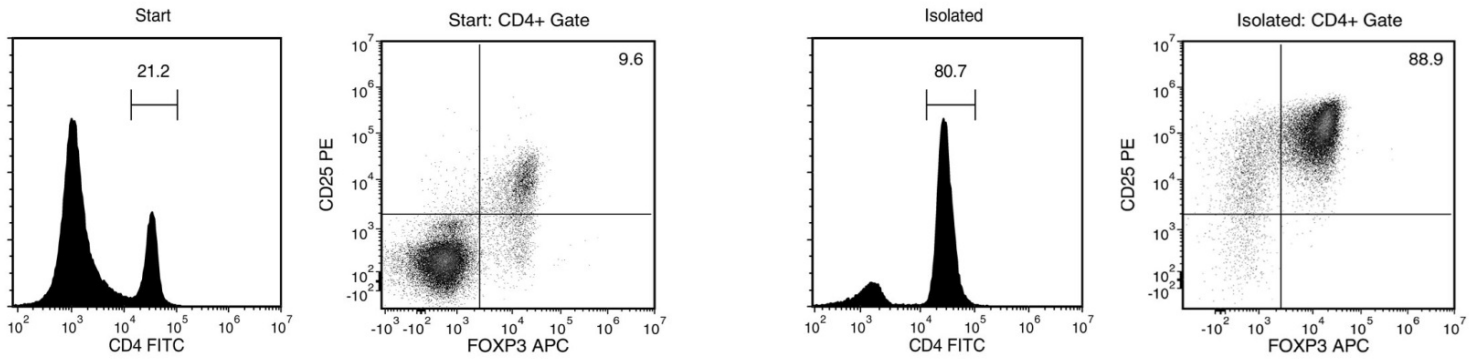

Figure 1. Typical EasySep™ Mouse CD4+CD25+ Regulatory T Cell Isolation Profile

Starting with mouse splenocytes, the regulatory T cell content (CD4+CD25+FOXP3+) of the isolated fraction typically ranges from 70 - 93%. In the above example, the purities of the start and final isolated fractions are 2% and 72%, respectively.