

# EasySep™ Mouse CD25 Regulatory T Cell Positive Selection Kit

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

Catalog #18782

Catalog #18782RF RoboSep™

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## Description

Isolate highly purified CD25+ cells from mouse splenocytes or other single-cell suspensions by immunomagnetic positive selection. When using single-cell suspensions from other tissue types, this kit may require optimization.

- Fast and easy-to-use
- Up to 93% purity
- No columns required

This kit targets CD25+ cells for positive selection with a PE-labeled antibody recognizing the CD25 surface marker. Desired cells are labeled with antibodies and magnetic particles and separated without columns using an EasySep™ magnet. Unwanted cells are simply poured off, while desired cells remain in the tube. Isolated cells are immediately available for downstream applications, such as flow cytometry, culture, or DNA/RNA extraction.

If isolating CD4+CD25- T cells from the same sample, contact us at [techsupport@stemcell.com](mailto:techsupport@stemcell.com) before starting the experiment to obtain a revised protocol using EasySep™ Mouse CD4+ T Cell Isolation Kit (Catalog #19852).

## Component Descriptions

COMPONENT NAME	COMPONENT #	QUANTITY	STORAGE	SHELF LIFE	FORMAT
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, 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™ 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

### SPLEEN

Disrupt spleen in recommended medium. Remove aggregates and debris by passing cell suspension through a 70 µm mesh nylon strainer (e.g. Catalog #27215). 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 containing 2% fetal bovine serum (FBS) with 1 mM EDTA. 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 CD25 Regulatory T Cell Positive Selection 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 10 <sup>8</sup> cells/mL 0.5 - 2 mL	1 x 10 <sup>8</sup> cells/mL 1 - 8 mL
	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)
2	Add FcR blocker to sample.	50 µL/mL of sample	50 µL/mL of sample
	Mix and incubate.	RT for 5 minutes	RT for 5 minutes
3	Add Positive Selection 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	Add PE Selection Cocktail to sample. NOTE: Do not vortex cocktail.	20 µL/mL of sample	20 µL/mL of sample
	Mix and incubate.	RT for 5 minutes	RT for 5 minutes
5	Vortex RapidSpheres™. NOTE: Particles should appear evenly dispersed.	30 seconds	30 seconds
6	Add RapidSpheres™ to sample.	20 µL/mL of sample	20 µL/mL of sample
	Mix and incubate.	RT for 5 minutes	RT for 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 5 minutes	RT for 5 minutes
8	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
9	Repeat steps as indicated.	Steps 7 and 8, three more times (total of 4 x 5-minute separations)	Steps 7 and 8, three more times (total of 4 x 5-minute separations)
10	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 CD25 Regulatory T Cell Positive Selection 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 0.5 - 1.5 mL	1 x 10 <sup>8</sup> cells/mL 1 - 8 mL
	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)
2	Add FcR blocker to sample.	50 µL/mL of sample	50 µL/mL of sample
	Mix and incubate.	RT for 5 minutes	RT for 5 minutes
3	Add Positive Selection 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	Add PE Selection Cocktail to sample. NOTE: Do not vortex cocktail.	20 µL/mL of sample	20 µL/mL of sample
	Mix and incubate.	RT for 5 minutes	RT for 5 minutes
5	Vortex RapidSpheres™. NOTE: Particles should appear evenly dispersed.	30 seconds	30 seconds
6	Add RapidSpheres™ to sample.	20 µL/mL of sample	20 µL/mL of sample
	Mix and incubate.	RT for 5 minutes	RT for 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 15 minutes	RT for 15 minutes
8	Carefully pipette** (do not pour) off the supernatant.	Discard supernatant	Discard supernatant
9	Repeat steps as indicated.	Steps 7 and 8, two more times (total of 3 x 15-minute separations)	Steps 7 and 8, two more times (total of 3 x 15-minute separations)
10	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 (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 CD25 Regulatory T Cell Positive Selection Kit Protocol**

STEP	INSTRUCTIONS	RoboSep™ (Catalog #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 sample to required tube.	14 mL (17 x 95 mm) polystyrene round-bottom tube (e.g. Catalog #38008)	
2	Add FcR blocker to sample.	50 µL/mL of sample	
	Mix and incubate.	RT for 5 minutes	
3	Select protocol.	Mouse CD25 Regulatory T Cell Positive Selection 18782	
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. 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	

## Notes and Tips

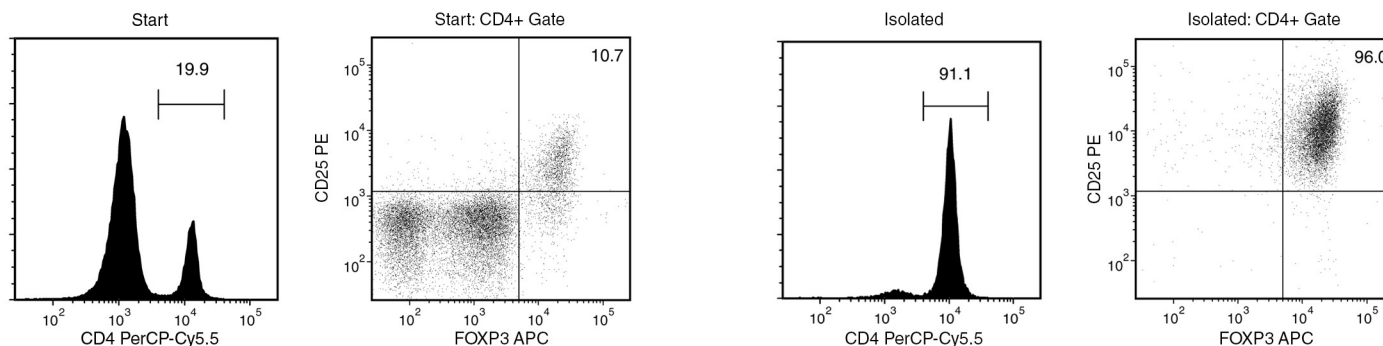
### ASSESSING PURITY

For purity assessment of regulatory T cells (CD4+CD25+FOXP3+) by flow cytometry, use the following fluorochrome-conjugated antibodies:

- Anti-Mouse CD4 Antibody, Clone RM4-5 (Catalog #60017), and
- Anti-mouse FOXP3 antibody

NOTE: The CD25+ cells have already been PE-labeled.

## Data



Starting with mouse splenocytes, the CD25 regulatory T cell content (CD4+CD25+FOXP3+) of the isolated fraction typically ranges from 80 - 93%. In the above example, the purities of the start and final isolated fractions are 2.1% and 87.5%, respectively.

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