EasySep™ Release Mouse CD4+CD304+ Regulatory T Cell Isolation Kit

For processing 1 x 10^9 cells

Catalog #100-1570
Catalog #100-1564 RoboSep™

Positive Selection

Document #10000027571 | Version 03

STEMCELL™ TECHNOLOGIES
Scientists Helping Scientists™ | WWW.STEMCELL.COM
TOLL FREE PHONE 1 800 667 0322 • PHONE +1 604 877 0713
INFO@STEMCELL.COM • TECHSUPPORT@STEMCELL.COM
FOR GLOBAL CONTACT DETAILS VISIT OUR WEBSITE

Description

Isolate CD4+CD304+ regulatory T cells (Tregs) from single-cell suspensions of mouse splenocytes or lymph nodes by immunomagnetic positive selection. When using single-cell suspensions from other tissue types, this kit may require optimization.

- Highly purified (up to 94%) mouse CD4+CD304+ Tregs isolated in less than 45 minutes
- No-wash removal of EasySep™ Releasable RapidSpheres™
- Optional isolation of CD4+CD304- responder T cells from the same sample

First, CD304+ cells are isolated by column-free immunomagnetic positive selection using EasySep™ Releasable RapidSpheres™. Then, bound magnetic particles are removed from the EasySep™-isolated CD304+ cells, and unwanted non-Tregs are targeted for depletion. The final isolated fraction contains highly purified CD4+CD304+ cells that express high levels of Foxp3 and CD25 and are immediately ready for downstream applications, such as flow cytometry, cell culture, or DNA/RNA extraction. By not targeting CD25, this kit does not interfere with IL-2 signaling in Tregs. An optional protocol allows for the isolation of CD4+CD304- responder T cells in parallel for use in functional studies.

Following cell isolation with this EasySep™ kit, antibody complexes remain bound to the cell surface and may interact with Brilliant Violet™ antibody conjugates, polyethylene glycol-modified proteins, or chemically related ligands.

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 CD304 Positive Selection Cocktail	300-1042	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™ Release PE Positive Selection Cocktail	300-1052	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™ Releasable RapidSpheres™ 50201	50201	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™ 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 water.
EasySep™ Release Buffer (Concentrate)	20165	3 x 1 mL	Store at 2 - 8°C. Do not freeze.	Stable until expiry date (EXP) on label.	A buffer for release of Releasable RapidSpheres™ from cells following positive selection.
EasySep™ Mouse FcR Blocker	18730	1 x 0.2 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.

RoboSep™ Release Mouse CD4+CD304+ Regulatory T Cell Isolation Kit is supplied with EasySep™ EasyTube™-14 (Catalog #20128) for optimal performance. The use of EasySep™ EasyTube™-14 is not required when performing a manual separation.

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 or LYMPH NODE

Disrupt spleen or lymph node 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++ and Mg++.

Directions for Use – Manual EasySep™ Protocols

See page 2 for Sample Preparation and Recommended Medium. Refer to Tables 1 and 2 for detailed instructions regarding the EasySep™ regulatory T cell isolation procedure. Refer to Tables 3 and 4 for the optional responder T cell enrichment protocol for each magnet.

Table 1. EasySep™ Release Mouse CD4+CD304+ Regulatory T Cell Isolation Kit Protocol

		EASYSEP™ MAGNETS	
STEP	INSTRUCTIONS	 **EasySep™ (Catalog #18000)**	**"The Big Easy" (Catalog #18001)** 
1	**Dilute Release Buffer (Concentrate) to prepare release buffer (1X).**	Dilute 1 in 40 with recommended medium. NOTE: Release buffer (1X) must be prepared on the day of use. Refer to step 15 for required volume.	Dilute 1 in 40 with recommended medium. NOTE: Release buffer (1X) must be prepared on the day of use. Refer to step 15 for required volume.
2	**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 0.5 - 8 mL
3	**Add Mouse FcR Blocker to sample.**	20 µL/mL of sample	20 µL/mL of sample
4	**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)
5	**Add CD4+ T Cell Isolation Cocktail to sample.** **NOTE: Do not vortex cocktail.**	50 µL/mL of sample	50 µL/mL of sample
6	**Add CD304 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 5 minutes	RT for 5 minutes
7	**Add Release PE 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 3 minutes	RT for 3 minutes
8	**Vortex Releasable RapidSpheres™.** **NOTE: Particles should appear evenly dispersed.**	30 seconds	30 seconds
Continue to step 9, next page		Continue to step 9, next page	Continue to step 9, next page

		EASYSEP™ MAGNETS	
STEP	INSTRUCTIONS (CONTINUED)	 **EasySep™ (Catalog #18000)**	**"The Big Easy" (Catalog #18001)** 
9	Add Releasable RapidSpheres™ to sample.	75 µL/mL of sample NOTE: Two different particles are provided in this kit. Ensure that Releasable RapidSpheres™ are used in this step.	75 µL/mL of sample NOTE: Two different particles are provided in this kit. Ensure that Releasable RapidSpheres™ are used in this step.
	Mix and incubate.	RT for 3 minutes	RT for 3 minutes
10	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
11	Pick up the magnet, and in one continuous motion invert the magnet and tube,* pouring the supernatant into a new tube.	Use a new 5 mL tube. Set aside supernatant for isolating CD4+CD304- responder T cells (Table 3) if desired.	Use a new 14 mL tube. Set aside supernatant for isolating CD4+CD304- responder T cells (Table 3) if desired.
12	Remove the tube from the magnet and 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
13	Pick up the magnet, and in one continuous motion invert the magnet and tube,* pouring off the supernatant.	Discard supernatant	Discard supernatant
14	Repeat steps as indicated.	Steps 12 and 13, two more times (total of 4 x 5-minute separations)	Steps 12 and 13, two more times (total of 4 x 5-minute separations)
15	Remove the tube from the magnet and add release buffer (1X) 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
	Incubate.	RT for 3 minutes	RT for 3 minutes
16	Vortex Streptavidin RapidSpheres™. NOTE: Particles should appear evenly dispersed.	30 seconds	30 seconds
17	Add Streptavidin RapidSpheres™ to sample.	10 µL/mL of sample (based on original volume from step 2) NOTE: Two different particles are provided in this kit. Ensure that Streptavidin RapidSpheres™ are used in this step.	10 µL/mL of sample (based on original volume from step 2) 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
Continue to step 18, next page		Continue to step 18, next page	Continue to step 18, next page

		EASYSEP™ MAGNETS	
STEP	INSTRUCTIONS (CONTINUED)	 EasySep™ (Catalog #18000)	"The Big Easy" (Catalog #18001) 
18	Place the tube (without lid) into the magnet and incubate.	RT for 5 minutes	RT for 5 minutes
19	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 (in the new tube) are ready for use	Isolated cells (in the new tube) 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™ Release Mouse CD4+CD304+ Regulatory T Cell Isolation Kit Protocol

		EASYSEP™ MAGNETS	
STEP	INSTRUCTIONS	 EasyEights™ (Catalog #18103)	
		5 mL tube	14 mL tube
1	Dilute Release Buffer (Concentrate) to prepare release buffer (1X).	Dilute 1 in 40 with recommended medium. NOTE: Release buffer (1X) must be prepared on the day of use. Refer to step 15 for required volume.	Dilute 1 in 40 with recommended medium. NOTE: Release buffer (1X) must be prepared on the day of use. Refer to step 15 for required volume.
2	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 0.5 - 8 mL
3	Add Mouse FcR Blocker to sample.	20 µL/mL of sample	20 µL/mL of sample
4	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)
5	Add CD4+ T Cell Isolation Cocktail to sample. NOTE: Do not vortex cocktail.	50 µL/mL of sample	50 µL/mL of sample
6	Add CD304 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 5 minutes	RT for 5 minutes
7	Add Release PE 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 3 minutes	RT for 3 minutes
8	Vortex Releasable RapidSpheres™. NOTE: Particles should appear evenly dispersed.	30 seconds	30 seconds
Continue to step 9, next page		Continue to step 9, next page	Continue to step 9, next page

STEP	INSTRUCTIONS (CONTINUED)	EASYSEP™ MAGNETS EasyEights™ (Catalog #18103) 5 mL tube	EASYSEP™ MAGNETS EasyEights™ (Catalog #18103) 14 mL tube
9	Add Releasable RapidSpheres™ to sample.	75 µL/mL of sample NOTE: Two different particles are provided in this kit. Ensure that Releasable RapidSpheres™ are used in this step.	75 µL/mL of sample NOTE: Two different particles are provided in this kit. Ensure that Releasable RapidSpheres™ are used in this step.
	Mix and incubate.	RT for 3 minutes	RT for 3 minutes
10	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 10 minutes	RT for 10 minutes
11	Carefully pipette** (do not pour) the supernatant into a new tube.	Use a new 5 mL tube. Set aside supernatant for isolating CD4+CD304- responder T cells (Table 4) if desired.	Use a new 14 mL tube. Set aside supernatant for isolating CD4+CD304- responder T cells (Table 4) if desired.
12	Remove the tube from the magnet and 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 10 minutes	RT for 10 minutes
13	Carefully pipette** (do not pour) the supernatant into a new tube.	Discard supernatant	Discard supernatant
14	Repeat steps as indicated.	Steps 12 and 13, one more time (total of 3 x 10-minute separations)	Steps 12 and 13, one more time (total of 3 x 10-minute separations)
15	Remove the tube from the magnet and add release buffer (1X) 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
	Incubate.	RT for 3 minutes	RT for 3 minutes
16	Vortex Streptavidin RapidSpheres™. NOTE: Particles should appear evenly dispersed.	30 seconds	30 seconds
17	Add Streptavidin RapidSpheres™ to sample.	10 µL/mL of sample (based on original volume from step 2) NOTE: Two different particles are provided in this kit. Ensure that Streptavidin RapidSpheres™ are used in this step.	10 µL/mL of sample (based on original volume from step 2) 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
18	Place the tube (without lid) into the magnet and incubate.	RT for 10 minutes	RT for 10 minutes
19	Carefully pipette** (do not pour) the enriched cell suspension into a new tube.	Isolated cells (in the new tube) are ready for use	Isolated cells (in the new tube) are ready for use

** 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]).

Table 3. Optional: Mouse CD4+CD304- Responder T Cell Enrichment Protocol

		EASYSEP™ MAGNETS	
STEP	INSTRUCTIONS	 **EasySep™ (Catalog #18000)**	**"The Big Easy" (Catalog #18001)** 
1	**Ensure cells are placed in the required tube.**	Supernatant from Table 1, step 11 must be in a 5 mL (12 x 75 mm) polystyrene round-bottom tube (e.g. Catalog #38007)	Supernatant from Table 1, step 11 must be in a 14 mL (17 x 95 mm) polystyrene round-bottom tube (e.g. Catalog #38008)
2	**Vortex Streptavidin RapidSpheres™.** NOTE: Particles should appear evenly dispersed.	30 seconds	30 seconds
3	**Add Streptavidin RapidSpheres™ to sample.**	75 µL/mL of sample (based on original volume from Table 1, step 2) NOTE: Two different particles are provided in this kit. Ensure that Streptavidin RapidSpheres™ are used in this step.	75 µL/mL of sample (based on original volume from Table 1, step 2) 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
4	**Place the tube (without lid) into the magnet and incubate.**	RT for 5 minutes	RT for 5 minutes
5	**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 (in the new tube) are ready for use	Isolated cells (in the new tube) are ready for use

*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 4. Optional: Mouse CD4+CD304- Responder T Cell Enrichment Protocol

		EASYSEP™ MAGNETS	
STEP	INSTRUCTIONS	 **EasyEights™ (Catalog #18103)**	
		5 mL tube	**14 mL tube**
1	**Ensure cells are placed in the required tube.**	Supernatant from Table 2, step 11 must be in a 5 mL (12 x 75 mm) polystyrene round-bottom tube (e.g. Catalog #38007)	Supernatant from Table 2, step 11 must be in a 14 mL (17 x 95 mm) polystyrene round-bottom tube (e.g. Catalog #38008)
2	**Vortex Streptavidin RapidSpheres™.** NOTE: Particles should appear evenly dispersed.	30 seconds	30 seconds
3	**Add Streptavidin RapidSpheres™ to sample.**	75 µL/mL of sample (based on original volume from Table 2, step 2) NOTE: Two different particles are provided in this kit. Ensure that Streptavidin RapidSpheres™ are used in this step.	75 µL/mL of sample (based on original volume from Table 2, step 2) 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
4	**Place the tube (without lid) into the magnet and incubate.**	RT for 10 minutes	RT for 10 minutes
5	**Carefully pipette** (do not pour) the enriched cell suspension into a new tube.**	Isolated cells (in the new tube) are ready for use	Isolated cells (in the new tube) are ready for use

**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 2 for Sample Preparation and Recommended Medium. Refer to Tables 5 and 6 for detailed instructions regarding the RoboSep™ procedure.

Table 5. RoboSep™ Release Mouse CD4+CD304+ Regulatory 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 1 - 8 mL
2	Add Mouse FcR Blocker to sample.	20 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.	• Release Mouse CD4+CD304+ Treg Positive Selection 100-1564 - small volume (< 4 mL) • Release Mouse CD4+CD304+ Treg Positive Selection 100-1564 - large volume (4 - 8 mL)
5	Vortex Releasable RapidSpheres™ and Streptavidin RapidSpheres™. NOTE: Particles should appear evenly dispersed.	30 seconds
6	Load the carousel.	Follow on-screen prompts NOTE: When prompted to load a separation tube, place an EasySep™ EasyTube™-14 into the magnet.
	Start the protocol.	Press the green “Run” button
7	Unload the carousel when the run is complete.	Isolated cells are ready for use

Table 6. Optional: RoboSep™ Release Mouse CD4+CD304+ Regulatory T Cell Isolation Kit with Responder T Cells 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 Mouse FcR Blocker to sample.	20 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.	• Release Mouse CD4+CD304+ Treg Positive Selection and Tresp 100-1564 - small volume (< 4 mL) • Release Mouse CD4+CD304+ Treg Positive Selection and Tresp 100-1564 - large volume (4 - 8 mL)
5	Vortex Releasable RapidSpheres™ and Streptavidin RapidSpheres™. NOTE: Particles should appear evenly dispersed.	30 seconds
6	Load the carousel.	Follow on-screen prompts NOTE: When prompted to load a separation tube in the first quadrant, place an EasySep™ EasyTube™-14 into the magnet. NOTE: When prompted to load a separation tube in the second quadrant, place a 14 mL (17 x 95 mm) polystyrene round-bottom tube (e.g. Catalog #38008).
	Start the protocol.	Press the green “Run” button
7	Unload the carousel when the run is complete.	Isolated cells are ready for use

Notes and Tips

EASYSEP™ RELEASE BUFFER

EasySep™ Release Buffer (Concentrate) is supplied as a 40X concentrate; release buffer (1X) must be prepared on the day of use. To prepare release buffer (1X), dilute an appropriate volume 1 in 40 with cold recommended medium. Refer to step 15 (Tables 1 or 2) for required volume. Keep diluted release buffer cold until use.

ASSESSING PURITY

For purity assessment of CD4+CD304+ regulatory T cells by flow cytometry, use the following fluorochrome-conjugated antibody clones:

- Anti-Mouse CD4 Antibody, Clone RM4-5 (Catalog #60017), and
- Anti-Mouse CD45 Antibody, Clone 30-F11 (Catalog #60030), and
- Anti-Mouse CD25, Clone PC61.5 (Catalog #60009) (optional), and
- Anti-Mouse FOXP3 antibody, clone FJK-16s (optional)

NOTE: The positively selected CD304+ cells have already been PE-labeled, so their purity can be assessed directly by flow cytometry.

NOTE: Brilliant Violet™ antibody conjugates should be carefully titrated on EasySep™ Release-isolated cells prior to analysis by flow cytometry or fluorescence microscopy. For purity assessment with Brilliant Violet™ antibody conjugates, use of BD Horizon Brilliant™ Stain Buffer is recommended to reduce non-specific interactions. For more information, refer to the manufacturer's instructions or contact us at techsupport@stemcell.com.

Data

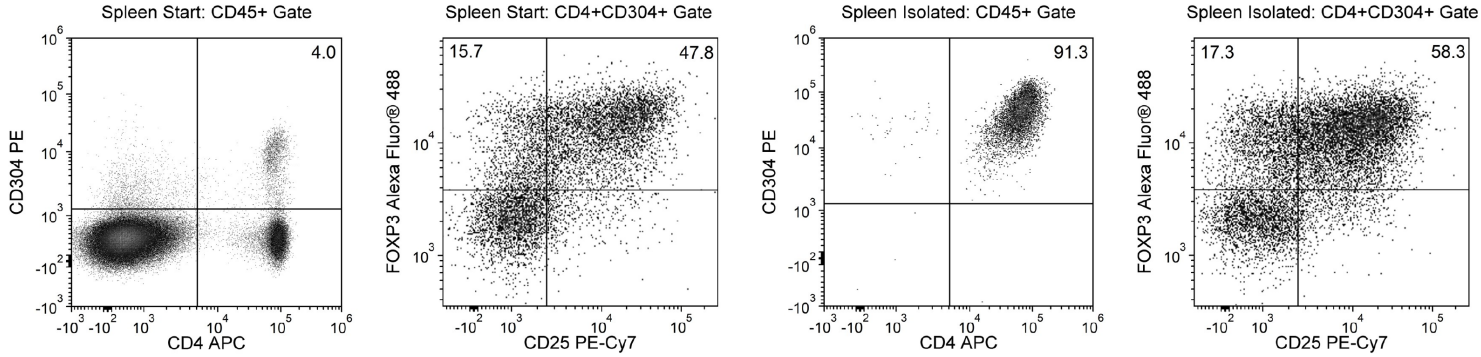

Figure 1. Isolation of CD4+CD304+ Regulatory T Cells from Mouse Splenocytes

Starting with naïve mouse splenocytes, the CD4+CD304+ T cell content of the final isolated fraction is typically 90.7 ± 3.0% and 64.2 ± 7.2% for CD4+CD304+CD25+FOXP3+ cells (mean ± SD using the "Big Easy" EasySep™ Magnet). In the above example, the purities of the start and final isolated fractions are 4.0% and 91.3% for CD4+CD304+ cells and 1.9% and 53.2% for CD4+CD304+CD25+FOXP3+ cells, respectively.

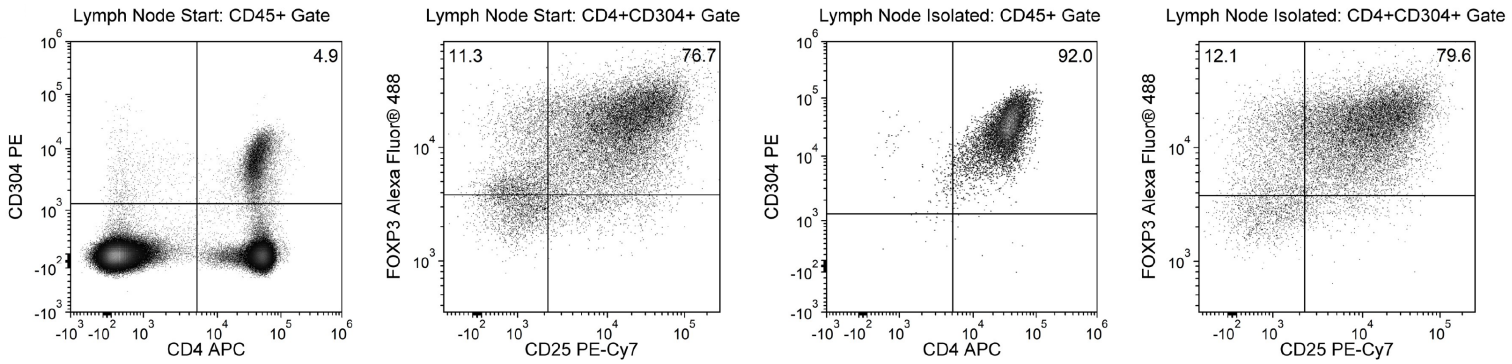

Figure 2. Isolation of CD4+CD304+ Regulatory T Cells from Mouse Lymph Nodes

Starting with naïve mouse lymph nodes, the CD4+CD304+ T cell content of the final isolated fraction is typically 92.1 ± 0.7% and 78.1 ± 1.4% for CD4+CD304+CD25+FOXP3+ cells (mean ± SD using the "Big Easy" EasySep™ Magnet). In the above example, the purities of the start and final isolated fractions are 4.9% and 92.0% for CD4+CD304+ cells and 3.8% and 73.2% for CD4+CD304+CD25+FOXP3+ cells, respectively.

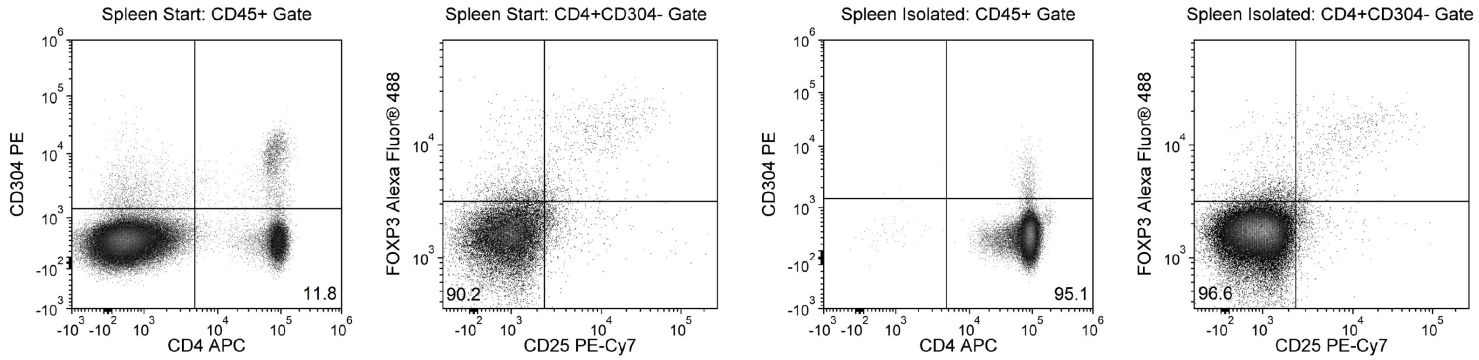

Figure 3. Isolation of CD4+CD304- Responder T Cells from Mouse Splenocytes

Starting with naïve mouse splenocytes, the CD4+CD304- responder T cell content of the final isolated fraction is typically 94.1 ± 2.9% and 90.6 ± 3.0% for CD4+CD304-CD25-FOXP3- cells (mean ± SD using the "Big Easy" EasySep™ Magnet). In the above example, the purities of the start and final isolated fractions are 11.8% and 95.1% for CD4+CD304- cells and 10.6% and 91.9% for CD4+CD304-CD25-FOXP3- cells, respectively.