EasySep™ Human CD4+CD127lowCD25+ Regulatory T Cell Isolation Kit

For processing 1 x 10^10 cells using the Easy 250 EasySep™ Magnet

Catalog #100-1136

Positive Selection

Document #10000023454 | Version 02

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Description

Isolate human CD4+CD127lowCD25+ regulatory T cells (Tregs) from fresh human peripheral blood mononuclear cells (PBMCs) or leukapheresis samples.

- No-wash removal of EasySep™ Releasable RapidSpheres™
- Optional isolation of CD4+CD25- responder T cells from the same sample

First, CD25+ cells are isolated by column-free immunomagnetic positive selection using EasySep™ Releasable RapidSpheres™. Then, bound magnetic particles are removed from the EasySep™-isolated CD25+ cells and unwanted non-Tregs are targeted for depletion. The final isolated fraction contains highly purified CD4+CD127lowCD25+ cells that express high levels of FOXP3 and are immediately ready for downstream applications. An optional protocol allows for the isolation of CD4+CD25- 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 other chemically related ligands.

NOTE: This is the Product Information Sheet (PIS) for isolating Tregs using the Easy 250 EasySep™ Magnet (Catalog #100-0821). If using other magnets, refer to the applicable PIS, available at www.stemcell.com or contact us to request a copy.

Component Descriptions

COMPONENT NAME	COMPONENT #	QUANTITY	STORAGE	SHELF LIFE	FORMAT
EasySep™ Human CD25 Positive Selection Cocktail	300-0814	1 x 10 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. Includes an Fc receptor blocking antibody.
EasySep™ Human CD127high Depletion Cocktail	300-0815	1 x 10 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™ Human CD4+ T Cell Enrichment Cocktail	300-0816	1 x 10 mL	Store at 2 - 8°C. Do not freeze.	Stable until expiry date (EXP) on label.	A combination of monoclonal antibodies in PBS.
EasySep™ Releasable RapidSpheres™ 50201	300-0817	1 x 10 mL	Store at 2 - 8°C. Do not freeze.	Stable until expiry date (EXP) on label.	A suspension of magnetic particles in water.
EasySep™ Dextran RapidSpheres™ 50103	300-0818	1 x 5 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*	100-1159	1 x 10 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.

BSA - bovine serum albumin; PBS - phosphate-buffered saline
* EasySep™ Release Buffer is ready for use; dilution is not required.

Components may be shipped at room temperature (15 - 25°C) but should be stored as indicated above.

Sample Preparation

For available fresh samples, see www.stemcell.com/primarycells.

NOTE: Working with fresh lysed leukapheresis samples is recommended for optimal performance. Alternatively, washed leukapheresis samples may be used (see below) for faster sample processing, but a reduction in performance may be observed.

LYSED LEUKAPHERESIS

1. Add an equal volume of Ammonium Chloride Solution (Catalog #07800) to the Leukopak (e.g. Human Peripheral Blood Leukopak, Fresh, Catalog #70500*). NOTE: If working with large volumes (> 150 mL), concentrate the Leukopak first by centrifuging at 300 x g for 10 minutes. Remove the supernatant and resuspend the cells in 1/10th of the original Leukopak volume with the recommended medium (e.g. for 300 mL of cells, resuspend in 30 mL of the recommended medium and add 30 mL of Ammonium Chloride Solution). For small volumes (≤ 150 mL), add Ammonium Chloride Solution directly to the Leukopak.
2. Incubate on ice for 15 minutes.
3. Centrifuge at 300 x g for 10 minutes at room temperature (15 - 25°C). Remove the supernatant.

4. Wash the cells by topping up the tube with the recommended medium. Centrifuge the cells at 120 x g for 10 minutes at room temperature with the brake off. Carefully remove the supernatant.
5. Repeat step 4 one or more times until most of the platelets have been removed (indicated by a clear supernatant).
6. Resuspend the cells at 5 x 10^7 cells/mL in the recommended medium.

* Some primary cell products are available only in select regions. Contact us at techsupport@stemcell.com for further information.

WASHED LEUKAPHERESIS

Wash the fresh peripheral blood leukapheresis sample (e.g. Human Peripheral Blood Leukopak, Fresh) by adding an equivalent volume of recommended medium or PBS containing 2% fetal bovine serum (FBS). Centrifuge at 300 x g for 10 minutes at room temperature (15 - 25°C). If platelet removal is necessary, centrifuge at 120 x g for 10 minutes with the brake off. Remove the supernatant and resuspend the cells at 5 x 10^7 cells/mL in recommended medium.

Recommended Medium

EasySep™ Buffer (Catalog #20144), or PBS containing 2% FBS and 1 mM EDTA. Medium should be free of Ca++ and Mg++.

Directions for Use – Manual EasySep™ Protocols

See pages 1 and 2 for Sample Preparation and Recommended Medium. Refer to Tables 1 and 2 for detailed instructions regarding the EasySep™ procedure.

Table 1. EasySep™ Human CD4+CD127lowCD25+ Regulatory T Cell Isolation Kit Protocol

		EASYSEP™ MAGNETS
STEP	INSTRUCTIONS	**Easy 250 (Catalog #100-0821)** 
1	Prepare sample at the indicated cell concentration within the volume range.	5 x 10^7 cells/mL 40 - 220 mL
	Add sample to required flask.	T-75 cm^2 cell culture flask (i.e. Catalog #200-0500)
2	Add CD25 Positive Selection Cocktail to sample. NOTE: Do not vortex cocktail.	50 µL/mL of sample
	Mix and incubate.	RT for 5 minutes
3	Vortex Releasable RapidSpheres™. NOTE: Particles should appear evenly dispersed.	30 seconds
	Add Releasable RapidSpheres™ to sample and mix.	30 µL/mL of sample
4	Add CD4+ T Cell Enrichment Cocktail to sample and mix.	50 µL/mL of sample
	Mix and incubate (see Notes and Tips).	RT for 5 minutes
5	Add recommended medium to top up sample to the indicated volume. Mix by gently pipetting up and down 2 - 3 times.	• Top up to 100 mL for samples < 80 mL • Top up to 250 mL for samples ≥ 80 mL
	Place the flask (without cap) into the magnet and incubate.	RT for 10 minutes
6	Carefully pipette (do not pour) the supernatant into a new flask.	Use a new flask Set aside supernatant for isolating CD4+CD25- responder T cells (Table 2) if desired.
OPTIONAL: To increase the recovery of CD25+ cells, perform an additional magnetic separation (steps 7 - 10); this may reduce purity.		Steps 7 - 10 OR Proceed to step 11
7	Vortex Releasable RapidSpheres™. NOTE: Particles should appear evenly dispersed.	30 seconds
8	Add Releasable RapidSpheres™ to the supernatant in the new flask.	15 µL/mL of original sample volume
	Mix and incubate.	RT for 4 minutes
9	Carefully pipette (do not pour) the supernatant back into the flask, in the magnet and incubate.	RT for 10 minutes
10	Carefully pipette (do not pour) the supernatant into a new flask.	Use a new flask Set aside supernatant for isolating CD4+CD25- responder T cells (Table 2) if desired.
Continue to step 11, next page		Continue to step 11, next page

STEP	INSTRUCTIONS (CONTINUED)	EASYSEP™ MAGNETS **Easy 250 (Catalog #100-0821)** 
11	**Remove the flask 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 100 mL for samples < 80 mL • Top up to 250 mL for samples ≥ 80 mL
	Place the flask (without cap) into the magnet and incubate.	RT for 10 minutes
12	**Carefully pipette (do not pour) off the supernatant and discard supernatant.**	Discard supernatant
13	**Repeat steps as indicated.**	Steps 11 and 12, two more times (total of 4 x 10-minute separations)
14	**Remove the flask 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.**	Same volume as the original starting sample volume (i.e. same volume used in step 1)
15	**Add Release Buffer to sample.**	50 µL/mL of sample
	Mix.	Vigorously pipette up and down more than 5 times
16	**Add CD127high Depletion Cocktail to sample.** **NOTE: Do not vortex cocktail.**	50 µL/mL of sample
	Mix and incubate.	RT for 5 minutes
17	**Vortex Dextran RapidSpheres™.** **NOTE: Particles should appear evenly dispersed.**	30 seconds
18	**Add Dextran RapidSpheres™ to sample.**	2.4 µL/mL of sample
	Mix and incubate.	RT for 5 minutes
19	**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 100 mL for samples < 80 mL • Top up to 250 mL for samples ≥ 80 mL
	Place the flask (without lid) into the magnet and incubate.	RT for 10 minutes
20	**Carefully pipette (do not pour) the supernatant into a new tube or centrifuge bottles.**	Use a new tube or centrifuge bottle*
21	**Centrifuge sample; carefully aspirate and discard supernatant.**	Centrifuge at 300 x g for 10 minutes at RT with low brake
	Resuspend to the desired cell concentration using recommended medium.	Isolated cells are ready for use

RT - room temperature (15 - 25°C)
* e.g. 50 mL (30 x 115 mm) conical tube (Catalog #38010) or 225 mL centrifuge bottle (Corning Catalog #352075)

Table 2. Optional: Human CD4+CD25- Responder T Cell Enrichment Protocol

		EASYSEP™ MAGNETS
STEP	INSTRUCTIONS	**Easy 250 (Catalog #100-0821)**
1	**Ensure cells are placed in the required flask.**	Supernatant from Table 1 (step 6 or step 10) must be in a T-75 cm^2 cell culture flask (i.e. Catalog #200-0500)
2	**Vortex Dextran RapidSpheres™.** **NOTE: Particles should appear evenly dispersed.**	30 seconds
3	**Add Dextran RapidSpheres™ to sample.**	10.8 µL/mL of original sample volume (see Table 1, step 1)
	Mix and incubate.	RT for 5 minutes
4	**Place the flask (without cap) into the magnet and incubate.**	RT for 10 minutes
5	**Carefully pipette (do not pour) the supernatant into a new flask.**	Use a new flask
6	**Remove the flask from the magnet and place the new flask (without cap) into the magnet and incubate.**	RT for 10 minutes
7	**Carefully pipette (do not pour) the supernatant into a new tube or centrifuge bottle.**	Use a new tube or centrifuge bottle*
8	**Centrifuge sample; carefully aspirate and discard supernatant.**	Centrifuge at 300 x g for 10 minutes at RT with low brake
9	**Resuspend to the desired cell concentration using recommended medium.**	Isolated cells are ready for use

RT - room temperature (15 - 25°C)
* e.g. 50 mL (30 x 115 mm) conical tube (Catalog #38010) or 225 mL centrifuge bottle (Corning Catalog #352075)

Notes and Tips

- After the addition of the Cocktails and RapidSpheres™, mix the sample with a 25 mL or 50 mL serological pipette (e.g. Catalog #38005/38006).

  NOTE: Mixing can also be performed by rotating or gently agitating the flask. Cap the flask first to prevent spilling.

- To collect the supernatant, gently sweep the pipette back and forth along the midline of the T-75 cm^2 flask while aspirating. Avoid touching the sides of the flask. Switch to a 10 mL or smaller serological pipette to collect the residual supernatant. Removal of the residual supernatant is required in order to obtain high purity.

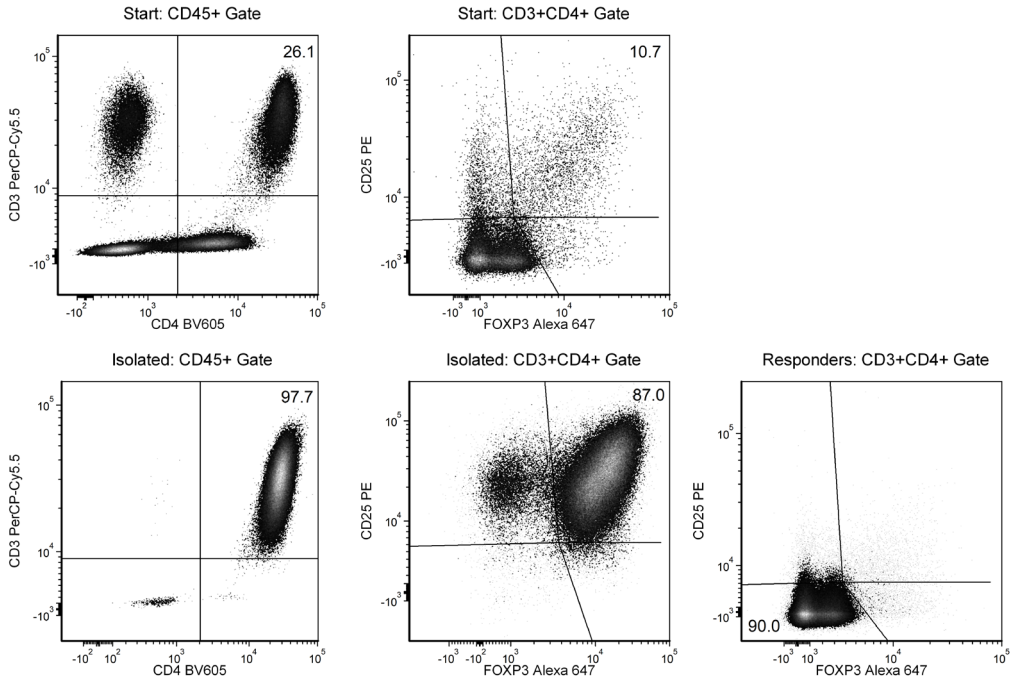
ASSESSING PURITY

EasySep™ Human CD25 Positive Selection Cocktail contains an anti-CD25 antibody clone that recognizes epitope B of the CD25 antigen and may block some anti-CD25 antibody clones used to assess purity by flow cytometry. For purity assessment of isolated cells by flow cytometry, use the following fluorochrome-conjugated antibody clones:

- Anti-Human CD3 Antibody, Clone UCHT1 (Catalog #60011; optional), and
- Anti-Human CD4 Antibody, Clone RPA-T4 (Catalog #100-0307), and
- Anti-Human CD25 Antibody, Clone 2A3 (Catalog #60153), which recognizes epitope A of the CD25 antigen, and
- Anti-Human CD45 Antibody, Clone HI30 (Catalog #60018; optional), and
- Anti-human CD127 antibody, clone hIL-7R-M21, and
- Anti-human FOXP3 antibody, clone 206D

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



Starting with washed or lysed leukapheresis samples, the regulatory T cell content (CD4+CD25+FOXP3+) of the isolated fraction is typically 78.4 ± 12.2% (mean ± SD). In the above example, the purities of the start and final isolated fractions are 2.8% and 85.0%, respectively. The responder T cell content (CD4+FOXP3-CD25-) is typically 68.8 ± 14.7%. In the above example, the purity of the final isolated responder T cell fraction is 90%.