EasySep™

Positive Selection

Catalog #18063

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

For processing 1 x 10^9 cells

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Document #28053 | Version 2_0_0

Description

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

- Highly purified human CD4+CD127lowCD25+ Tregs isolated in less than 1 hour
- 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.

Component Descriptions

COMPONENT NAME	COMPONENT #	QUANTITY	STORAGE	SHELF LIFE	FORMAT
EasySep™ Human CD25 Positive Selection Cocktail	18063C.1	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 and 0.1% BSA. Includes an Fc receptor blocking antibody.
EasySep™ Human CD4+ T Cell Enrichment Cocktail	19052C.2	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™ Human CD127high Depletion Cocktail	19233C	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 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 water.
EasySep™ Dextran RapidSpheres™ 50100	50100	2 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	20145	2 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.

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 available fresh and frozen samples, see www.stemcell.com/primarycells.

PERIPHERAL BLOOD

Prepare a PBMC suspension from whole blood by centrifugation over a density gradient medium (e.g. Lymphoprep™, Catalog #07801). For more rapid PBMC preparation, use the SepMate™ RUO (Catalog #86450/86415) or SepMate™ IVD* (Catalog #85450/85415) cell isolation tube.

If using previously frozen PBMCs, incubate the cells with DNase I Solution (Catalog #07900) at a concentration of 100 µg/mL at room temperature (15 - 25°C) for at least 15 minutes prior to labeling and separation. Filter aggregated suspensions through a 40 µm Cell Strainer (Catalog #27305) for optimal results.

After preparation, resuspend cells at 5 x 10^7 cells/mL in recommended medium.

* SepMate™ IVD is only available in select regions where it is registered as an In Vitro Diagnostic (IVD) device for the isolation of mononuclear cells (MNCs) from whole blood or bone marrow by density gradient centrifugation. In all other regions SepMate™ is available for research use only (RUO).

LEUKAPHERESIS (LEUKO PAK)

Wash the peripheral blood leukapheresis sample by adding an equivalent volume of recommended medium or PBS containing 2% fetal bovine serum (FBS). Centrifuge at 500 x g for 10 minutes at room temperature (15 - 25°C). 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 page 2 for Sample Preparation and Recommended Medium. Refer to Tables 1, 2, 3, and 4 for detailed instructions regarding the EasySep™ procedure for each magnet.

Table 1. EasySep™ Human CD4+CD127lowCD25+ Regulatory 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.	5 x 10^7 cells/mL 0.25 - 2 mL	5 x 10^7 cells/mL 0.5 - 6 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 CD25 Positive Selection Cocktail to sample.	50 µL/mL of sample	50 µL/mL of sample
	Mix and incubate.	RT for 5 minutes	RT for 5 minutes
3	Vortex Releasable RapidSpheres™. NOTE: Particles should appear evenly dispersed.	30 seconds	30 seconds
	Add Releasable RapidSpheres™ to sample and mix.	30 µL/mL of sample	30 µL/mL of sample
4	Add CD4+ T Cell Enrichment Cocktail to sample.	50 µL/mL of sample	50 µL/mL of sample
	Mix and incubate.	RT for 5 minutes	RT for 5 minutes
5	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
6	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+CD25- responder T cells (Table 3) if desired.	Use a new 14 mL tube. Set aside supernatant for isolating CD4+CD25- responder T cells (Table 3) if desired.
7	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
8	Pick up the magnet, and in one continuous motion invert the magnet and tube,* pouring off the supernatant.	Discard supernatant	Discard supernatant
9	Repeat steps as indicated.	Steps 7 and 8, two more times (total of 1 x 10-minute and 3 x 5-minute separations)	Steps 7 and 8, two more times (total of 1 x 10-minute and 3 x 5-minute separations)
Continue to step 10, next page		Continue to step 10, next page	Continue to step 10, next page

		EASYSEP™ MAGNETS	
STEP	INSTRUCTIONS (CONTINUED)	 **EasySep™ (Catalog #18000)**	**"The Big Easy" (Catalog #18001)** 
10	**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. Be sure to collect cells off the sides of the tube.**	Same volume as the original starting sample volume (i.e. same volume used in step 1)	Same volume as the original starting sample volume (i.e. same volume used in step 1)
11	**Add Release Buffer to sample.**	100 µL/mL of sample	100 µL/mL of sample
	Mix.	Vigorously pipette up and down more than 5 times	Vigorously pipette up and down more than 5 times
12	**Add CD127high Depletion Cocktail to sample.**	50 µL/mL of sample	50 µL/mL of sample
	Mix and incubate.	RT for 5 minutes	RT for 5 minutes
13	**Vortex Dextran RapidSpheres™.** **NOTE: Particles should appear evenly dispersed.**	30 seconds	30 seconds
14	**Add Dextran RapidSpheres™ to sample.**	10 µL/mL of sample	10 µL/mL of sample
	Mix and incubate.	RT for 5 minutes	RT for 5 minutes
15	**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 start sample ≤ 4 mL • Top up to 10 mL for start sample > 4 mL
	Place the tube (without lid) into the magnet and incubate.	RT for 5 minutes	RT for 5 minutes
16	**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™ Human CD4+CD127lowCD25+ Regulatory T Cell Isolation Kit Protocol

		EASYSEP™ MAGNETS		
		EasyEights™ (Catalog #18103)		
STEP	INSTRUCTIONS	5 mL tube	14 mL tube	Easy 50 (Catalog #18002)
1	Prepare sample at the indicated cell concentration within the volume range.	5 x 10^7 cells/mL 0.25 - 2 mL	5 x 10^7 cells/mL 0.5 - 6 mL	5 x 10^7 cells/mL 5 - 40 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)	50 mL (30 x 115 mm) conical tube (e.g. Catalog #38010)
2	Add CD25 Positive Selection Cocktail to sample.	50 µL/mL of sample	50 µL/mL of sample	50 µL/mL of sample
	Mix and incubate.	RT for 5 minutes	RT for 5 minutes	RT for 5 minutes
3	Vortex Releasable RapidSpheres™. NOTE: Particles should appear evenly dispersed.	30 seconds	30 seconds	30 seconds
	Add Releasable RapidSpheres™ to sample and mix.	30 µL/mL of sample	30 µL/mL of sample	30 µL/mL of sample
4	Add CD4+ T Cell Enrichment Cocktail to sample.	50 µL/mL of sample	50 µL/mL of sample	50 µL/mL of sample
	Mix and incubate.	RT for 5 minutes	RT for 5 minutes	RT for 5 minutes
5	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	• Top up to 25 mL for samples ≤ 20 mL • Top up to 50 mL for samples > 20 mL
	Place the tube (without lid) into the magnet and incubate.	RT for 10 minutes	RT for 10 minutes	RT for 10 minutes
6	Carefully pipette*** (do not pour) the supernatant into a new tube.	Use a new 5 mL tube. Set aside supernatant for isolating CD4+CD25- responder T cells (Table 4) if desired.	Use a new 14 mL tube. Set aside supernatant for isolating CD4+CD25- responder T cells (Table 4) if desired.	Use a new 50 mL tube. Set aside supernatant for isolating CD4+CD25- responder T cells (Table 4) if desired.
7	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	• Top up to 25 mL for samples ≤ 20 mL • Top up to 50 mL for samples > 20 mL
	Place the tube (without lid) into the magnet and incubate.	RT for 5 minutes	RT for 5 minutes	RT for 10 minutes
8	Carefully pipette** (do not pour) off the supernatant.	Discard supernatant	Discard supernatant	Discard supernatant
9	Repeat steps as indicated.	Steps 7 and 8, two more times (total of 1 x 10-minute and 3 x 5-minute separations)	Steps 7 and 8, two more times (total of 1 x 10-minute and 3 x 5-minute separations)	Steps 7 and 8, two more times (total of 4 x 10-minute separations)
Continue to step 10, next page		Continue to step 10, next page	Continue to step 10, next page	Continue to step 10, next page

		EASYSEP™ MAGNETS		
		EasyEights™ (Catalog #18103)		
STEP	INSTRUCTIONS (CONTINUED)	5 mL tube	14 mL tube	Easy 50 (Catalog #18002)
10	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. Be sure to collect cells off the sides of the tube.	Same volume as the original starting sample volume (i.e. same volume used in step 1)	Same volume as the original starting sample volume (i.e. same volume used in step 1)	Same volume as the original starting sample volume (i.e. same volume used in step 1)
11	Add Release Buffer to sample.	100 µL/mL of sample	100 µL/mL of sample	100 µL/mL of sample
	Mix.	Vigorously pipette up and down more than 5 times	Vigorously pipette up and down more than 5 times	Vigorously pipette up and down more than 5 times
12	Add CD127high Depletion Cocktail to sample.	50 µL/mL of sample	50 µL/mL of sample	50 µL/mL of sample
	Mix and incubate.	RT for 5 minutes	RT for 5 minutes	RT for 5 minutes
13	Vortex Dextran RapidSpheres™. NOTE: Particles should appear evenly dispersed.	30 seconds	30 seconds	30 seconds
14	Add Dextran RapidSpheres™ to sample.	10 µL/mL of sample	10 µL/mL of sample	20 µL/mL of sample
	Mix and incubate.	RT for 5 minutes	RT for 5 minutes	RT for 5 minutes
15	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	• Top up to 25 mL for samples ≤ 20 mL • Top up to 50 mL for samples > 20 mL
	Place the tube (without lid) into the magnet and incubate.	RT for 5 minutes	RT for 5 minutes	RT for 10 minutes
16	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	Isolated cells in the new tube are ready for use

RT - room temperature (15 - 25°C)

*** Collect the entire enriched cell suspension, 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: Human CD4+CD25- 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 6 must be in a 5 mL (12 x 75 mm) polystyrene round-bottom tube (e.g. Catalog #38007)	Supernatant from Table 1, step 6 must be in a 14 mL (17 x 95 mm) polystyrene round-bottom tube (e.g. Catalog #38008)
2	Vortex Dextran RapidSpheres™. NOTE: Particles should appear evenly dispersed.	30 seconds	30 seconds
3	Add Dextran RapidSpheres™ to sample and mix.	90 µL/mL of original sample volume (see Table 1, step 1)	90 µL/mL of original sample volume (see Table 1, step 1)
	Mix and incubate.	RT for 5 minutes	RT for 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 supernatant into a new tube.	Use a new 5 mL tube	Use a new 14 mL tube
6	Remove the tube from the magnet. Place the new tube containing the enriched cells (without lid) into the magnet and incubate for a second separation.	RT for 1 minute	RT for 1 minute
7	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 are ready for use	Isolated cells are ready for use

Table 4. Optional: Human CD4+CD25- Responder T Cell Enrichment Protocol

		EASYSEP™ MAGNETS		
		EasyEights™ (Catalog #18103)		
STEP	INSTRUCTIONS	 **5 mL tube**	**14 mL tube** 	**Easy 50 (Catalog #18002)** 
1	Ensure cells are placed in the required tube.	Supernatant from Table 2, step 6 must be in a 5 mL (12 x 75 mm) polystyrene round-bottom tube (e.g. Catalog #38007)	Supernatant from Table 2, step 6 must be in a 14 mL (17 x 95 mm) polystyrene round-bottom tube (e.g. Catalog #38008)	Supernatant from Table 2, step 6 must be in a 50 mL (30 x 115 mm) conical tube (e.g. Catalog #38010)
2	Vortex Dextran RapidSpheres™. NOTE: Particles should appear evenly dispersed.	30 seconds	30 seconds	30 seconds
3	Add Dextran RapidSpheres™ to sample.	90 µL/mL of original sample volume (see Table 2, step 1)	90 µL/mL of original sample volume (see Table 2, step 1)	180 µL/mL of original sample volume (see Table 2, step 1)
	Mix and incubate.	RT for 5 minutes	RT for 5 minutes	RT for 5 minutes
4	Place the tube (without lid) into the magnet and incubate.	RT for 5 minutes	RT for 5 minutes	RT for 10 minutes
5	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	Use a new 50 mL tube
6	Remove the tube from the magnet. Place the new tube containing the enriched cells (without lid) into the magnet and incubate for a second separation.	RT for 5 minute	RT for 5 minutes	RT for 10 minutes
7	Carefully pipette** (do not pour) the enriched cell suspension into a new tube.	Isolated cells are ready for use	Isolated cells are ready for use	Isolated cells are ready for use

Notes and Tips

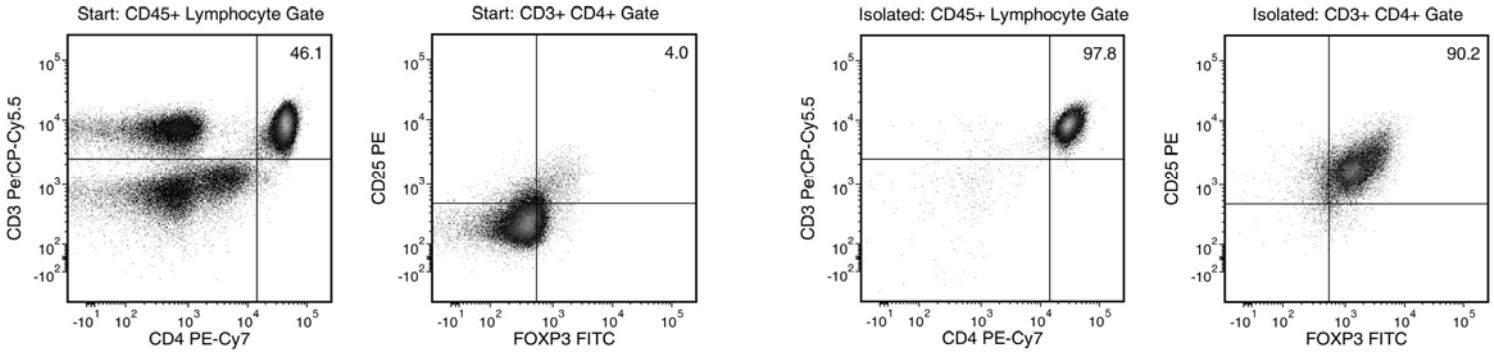
ASSESSING PURITY

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, 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 fresh or previously frozen PBMCs, the regulatory T cell content (CD4+CD25+FOXP3+) of the isolated fraction is typically 85.0 ± 4.8% (mean ± SD). In the above example, the purities of the start and final isolated fractions are 1.8% and 88.2%, respectively.