



EasySep™ Human CD25 Depletion Kit

Negative Selection

Catalog #17861

For processing 1×10^9 cells



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Description

Deplete CD25+ cells from fresh human peripheral blood mononuclear cells (PBMCs) or lysed leukapheresis samples by immunomagnetic depletion.

- Fast, easy-to-use and column-free
- Up to 1.3 log depletion of CD25+ cells

This kit targets CD25+ cells for removal with an antibody recognizing the CD25 surface marker. Unwanted cells are labeled with antibodies and magnetic particles, and separated without columns using an EasySep™ magnet. Desired cells are simply poured off, while unwanted cells remain in the tube. Isolated cells are immediately available for downstream applications such as flow cytometry, in vitro assays, or cell culture.

Component Descriptions

COMPONENT NAME	COMPONENT #	QUANTITY	STORAGE	SHELF LIFE	FORMAT
EasySep™ Human CD25 Depletion Cocktail	17861C	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. Includes an Fc receptor blocking antibody.
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.

PBS - phosphate-buffered saline

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

Sample Preparation

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.

After preparation, resuspend cells at 1×10^8 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.

LEUKAPHERESIS (LEUKO PAK)

If working with large volumes (> 150 mL), concentrate leukapheresis sample first by centrifuging at 500 x g for 10 minutes. Remove the supernatant and resuspend the cells in 1/10th of the original leukapheresis volume with recommended medium (e.g. for 300 mL of cells, resuspend in 30 mL of recommended medium). For small volumes (≤ 150 mL), add Ammonium Chloride Solution (Catalog #07800) directly to the leukapheresis sample.

1. Add an equal volume of Ammonium Chloride Solution to the leukapheresis sample.
2. Incubate on ice for 15 minutes.
3. Centrifuge at 500 x g for 10 minutes at room temperature (15 - 25°C). Remove the supernatant.
4. Wash the cells by topping up the tube with recommended medium. Centrifuge the cells at 150 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 1×10^8 cells/mL in recommended medium.

Recommended Medium

EasySep™ Buffer (Catalog #20144) or PBS containing 2% fetal bovine serum 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 Table 1 for detailed instructions regarding the EasySep™ procedure for each magnet.

Table 1. EasySep™ Human CD25 Depletion 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.	1 x 10 ⁸ cells/mL 0.5 - 2 mL	1 x 10 ⁸ cells/mL 1 - 8 mL
	Add sample to required tube.	5 mL (12 x 75 mm) polystyrene round-bottom tube (e.g. Corning Catalog #352058)	14 mL (17 x 100 mm) polystyrene round-bottom tube (e.g. Corning Catalog #352057)
2	Add Depletion Cocktail to sample.	100 µL/mL of sample	100 µL/mL of sample
	Mix and incubate.	RT for 10 minutes	RT for 10 minutes
3	Vortex RapidSpheres™.	30 seconds	30 seconds
4	Add RapidSpheres™ to sample.	200 µL/mL of sample	200 µL/mL of sample
	Mix and incubate.	RT for 3 minutes	RT for 3 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 2.5 mL for samples ≤ 2 mL • Top up to 5 mL for samples > 2 - 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
6	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
7	Remove the tube from the magnet and place the new tube (without lid) into the magnet and incubate for a second separation.	RT for 5 minutes	RT for 5 minutes
8	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

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.

Directions for Use – OPTIONAL Manual EasySep™ Protocols

EasySep™ Human CD25 Depletion Kit can also be used to positively select CD25+ cells. Refer to Table 2 for detailed instructions regarding the EasySep™ procedure for each magnet.

Table 2. Optional Positive Selection Protocol Using EasySep™ Human CD25 Depletion Kit

		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
	Add sample to required tube.	5 mL (12 x 75 mm) polystyrene round-bottom tube (e.g. Corning Catalog #352058)	14 mL (17 x 100 mm) polystyrene round-bottom tube (e.g. Corning Catalog #352057)
2	Add Depletion Cocktail to sample.	50 µL/mL of sample	50 µL/mL of sample
	Mix and incubate.	RT for 10 minutes	RT for 10 minutes
3	Vortex RapidSpheres™.	30 seconds	30 seconds
4	Add RapidSpheres™ to sample.	100 µL/mL of sample	100 µL/mL of sample
	Mix and incubate.	RT for 3 minutes	RT for 3 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 2.5 mL for samples ≤ 2 mL • Top up to 5 mL for samples > 2 - 4 mL • Top up to 10 mL for samples > 4 mL
	Place the tube (without lid) into the magnet and incubate.	RT for 3 minutes	RT for 3 minutes
6	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
7	Repeat steps as indicated	Steps 5 and 6, three more times (total of 4 x 3-minute separations)	Steps 5 and 6, three more times (total of 4 x 3-minute separations)
8	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.

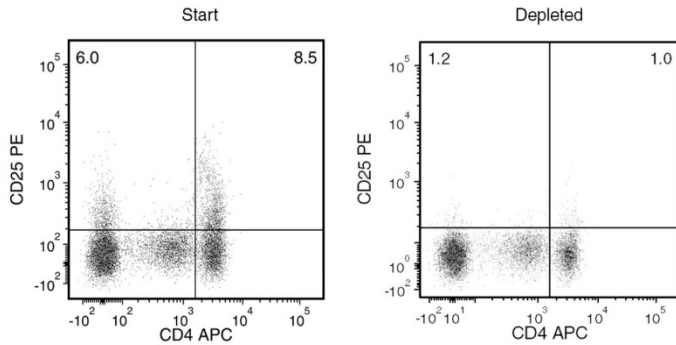
Notes and Tips

ASSESSING PURITY

EasySep™ Human CD25 Positive Selection Cocktail uses an anti-CD25 antibody clone which 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 residual CD25+ cells by flow cytometry use the following fluorochrome-conjugated antibodies:

- Anti-Human CD25 Antibody, Clone 2A3, PE (Catalog #60153), or clone BC96 which recognize epitope A of the CD25 antigen, and
- Anti-human CD4 antibody, clone RPA-T4

Data



Starting with fresh PBMCs, the CD25+ cell content of the depleted fraction typically ranges from 2 - 5%. In the above example, the purities of the start and final depleted fractions are 14.5% and 2.2%, respectively, with a 1.3 log depletion of CD25+ cells. Using the optional positive selection protocol, the CD25+ cell content of the isolated fraction typically ranges from 81 - 98% (data not shown) .

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