EasySep™ Human TCR Alpha/Beta Depletion Kit

Negative Selection

Catalog #17847

For processing 1 x 10^9 cells

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Description

Deplete human T cell receptor alpha/beta+ (TCRαß+) cells from leukapheresis samples or expanded TCRαß knockout cell cultures.

- Fast and easy-to-use
- No columns required
- Isolated cells are untouched

This kit targets TCRαß+ cells for removal with an antibody recognizing the TCRαß surface marker. Unwanted cells are labeled with antibody and magnetic particles, and separated without columns using an EasySep™ magnet. Desired cells are simply poured off into a new tube. Isolated cells are immediately available for downstream applications such as flow cytometry, culture, or cryopreservation.

Component Descriptions

COMPONENT NAME	COMPONENT #	QUANTITY	STORAGE	SHELF LIFE	FORMAT
EasySep™ Human TCR Alpha/Beta Depletion Cocktail	17847C	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™ 50103	50103	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

For generating a TCRαß knockout cell population, refer to the Technical Bulletin: Genome Editing of Human Primary T Cells (Document #27155), available at www.stemcell.com or contact us to request a copy.

EXPANDED TCRαß KNOCKOUT CELLS

Harvest cultured cells and remove the culture medium by centrifugation. Resuspend the cells at 5 x 10^7 cells/mL in recommended medium.

LEUKAPHERESIS

1. Add 3 parts Ammonium Chloride (Catalog #07800) to 1 part leukapheresis sample.
2. Incubate on ice for 15 minutes.
3. Centrifuge at 300 x g for 10 minutes at room temperature (15 - 25°C). Carefully remove the supernatant.
4. Wash the cells by topping up the tube with recommended medium. Centrifuge cells at 150 x g for 10 minutes at room temperature with the brake off. Carefully remove the supernatant.
5. Resuspend cells at 5 x 10^7 cells/mL in recommended medium.

Recommended Medium

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

Table 1. EasySep™ Human TCR Alpha/Beta 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.**	5 x 10^7 cells/mL 0.25 - 2 mL	5 x 10^7 cells/mL 0.5 - 8.5 mL
	Add sample to required tube.	5 mL (12 x 75 mm) polystyrene round-bottom tube (e.g. Catalog #38007)	14 mL (17 x 100 mm) polystyrene round-bottom tube (e.g. Catalog #38008)
2	**Add 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
3	**Vortex RapidSpheres™.** **NOTE: Particles should appear evenly dispersed.**	30 seconds	30 seconds
4	**Add RapidSpheres™ to sample.**	50 µL/mL of sample	50 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 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
6	**Pick up the magnet, and in one continuous motion invert the magnet and tube,* pouring the depleted 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.

Table 2. EasySep™ Human TCR Alpha/Beta Depletion Kit Protocol

		EASYSEP™ MAGNETS
STEP	INSTRUCTIONS	**Easy 50 (Catalog #18002)** 
1	Prepare sample at the indicated cell concentration within the volume range.	5 x 10^7 cells/mL 5 - 40 mL
	Add sample to required tube.	50 mL (30 x 115 mm) conical tube (e.g. Catalog #38010)
2	Add Depletion Cocktail to sample.	50 µL/mL of sample
	Mix and incubate.	RT for 10 minutes
3	Vortex RapidSpheres™. NOTE: Particles should appear evenly dispersed.	30 seconds
4	Add RapidSpheres™ to sample.	100 µL/mL of sample
	Mix and incubate.	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 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
6	Carefully pipette (do not pour) the depleted cell suspension into a new tube.	Use a new 50 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 10 minutes (total of 2 x 10-minute separations)
8	Carefully pipette (do not pour) the depleted cell suspension into a new tube.	Isolated cells are ready for use

RT - room temperature (15 - 25°C)

Notes and Tips

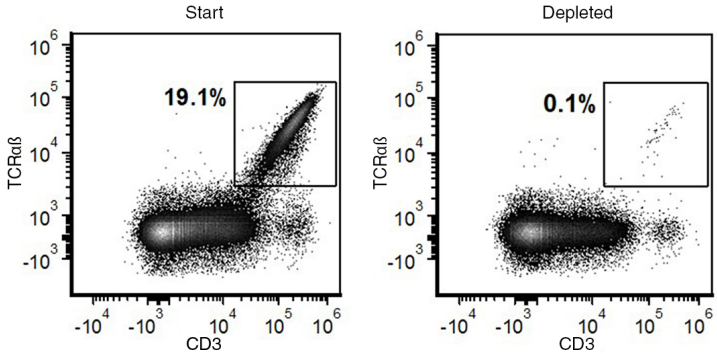
ASSESSING PURITY

For purity assessment of residual TCRαß+ cells by flow cytometry, use the following fluorochrome-conjugated antibody clones:

- Anti-human TCRαß antibody, clone IP26 (partially blocked), or
- Anti-human TCRαß antibody, clone T10B9.1A31 (partially blocked)

NOTE: The expression of endogenous TCRαß on human primary T cells can be disrupted using ArciTect™ CRISPR/Cas9-mediated gene modification. The TCRαß-knockout efficiency typically ranges from 60 - 90%. The expression level of TCRαß in the starting population from expanded TCRαß knockout cell culture varies with the TCRαß knockout efficiency.

Data



In the above example, the frequencies of CD3+TCRαß+ cells in the starting and depleted fractions are 19.1% and 0.1%, respectively.