EasySep™ Human TCR Alpha/Beta Depletion Kit

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

Catalog #100-1660

Negative Selection

Document #10000027825 | Version 01

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Description

Deplete human T cell receptor alpha/beta+ (TCRαß+) cells from fresh leukapheresis samples.

- 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 pipetted off. Isolated cells are immediately available for downstream applications, such as flow cytometry, culture, or cryopreservation.

NOTE: This is the Product Information Sheet (PIS) for depleting TCRαß+ cells 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 TCR Alpha/Beta Depletion Cocktail	300-1045	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™ Dextran RapidSpheres™ 50103	300-0380	2 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.

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 samples, see www.stemcell.com/primarycells.

NOTE: Working with fresh lysed leukapheresis samples is recommended for optimal performance.

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.

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 Table 1 for detailed instructions regarding the EasySep™ procedure.

Table 1. EasySep™ Human TCR Alpha/Beta Depletion Kit Protocol

STEP	INSTRUCTIONS	Easy 250 EasySep™ (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 (e.g. Catalog #200-0500)
2	Add Depletion Cocktail to sample. Note: Do not vortex cocktail.	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 (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 depleted cell suspension into a new flask.	Use a new T-75 cm^2 flask
7	Remove the flask from the magnet; place the new flask (without cap) 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 or centrifuge bottle*.	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

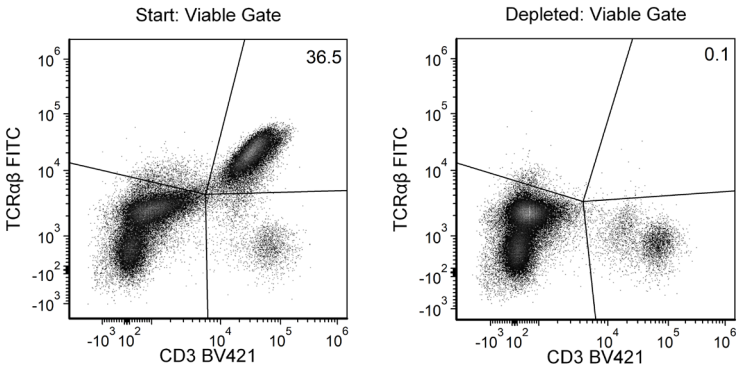
- After addition of the Cocktail and RapidSpheres™, mix the sample with a 25 mL or 50 mL serological pipette (e.g. Catalog #38005/38006).
  NOTE: Mixing can also be performed by rotating or gently agitating the flask. Cap the flask first to prevent spillage.
- To collect the supernatant, gently sweep 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.

ASSESSING PURITY

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

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

Data



In the above example, the frequencies of CD3+TCRαß+ cells in the starting and depleted fractions are 36.5% and 0.1%, respectively.