



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

Positive Selection

Catalog #18063RF

For processing 1 x 10⁹ cells



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Description

Isolate human CD4+CD127lowCD25+ regulatory T cells (Tregs) from fresh or previously frozen 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+ Tregs 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 RoboSep™ 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: For manual magnet separation, refer to the Product Information Sheet for EasySep™ Human CD4+CD127lowCD25+ Regulatory T Cell Isolation Kit (Document #28053), 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	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.
RoboSep™ Filter Tips	20125	2 x 8 Racks	Not Applicable	Not Applicable	Not Applicable
EasySep™ EasyTube™-14	20128	1 Bag	Not Applicable	Not Applicable	Not Applicable

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.

Notes and Tips

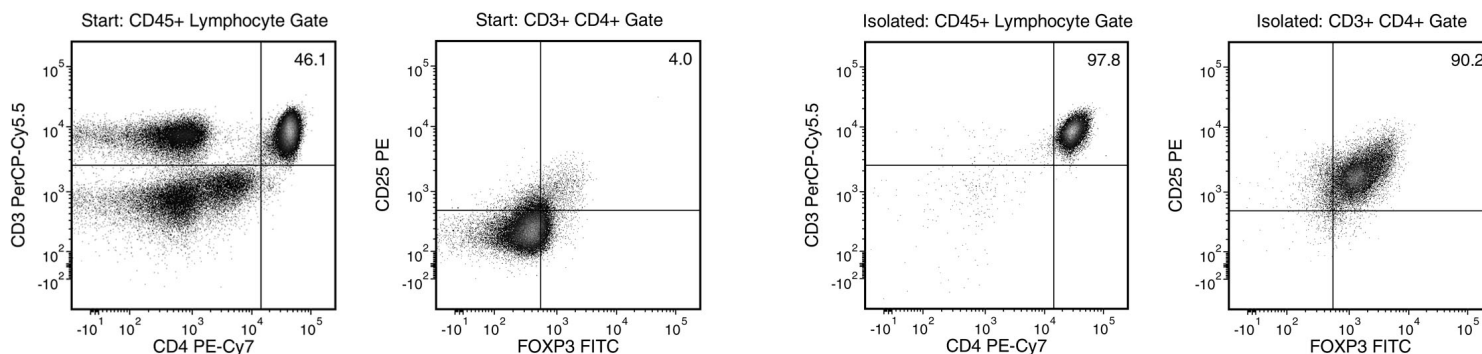
ASSESSING PURITY

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 Treg content (CD4+CD25+FOXP3+) of the isolated fraction is typically $85.0 \pm 4.8\%$ (mean \pm SD). In the above example, the purities of the start and final isolated fractions are 1.8% and 88.2%, respectively.

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