

Primary Cells

**Human Peripheral Blood
CD4+CD25+CD127lowFOXP3+ T Cells
(Tregs), Frozen**



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Catalog #200-0120
#200-0121

1 x 10⁶ cells
2 x 10⁶ cells

Product Description

Primary human CD4+CD25+CD127lowFOXP3+ T cells were isolated from peripheral blood mononuclear cells (PBMCs) using positive immunomagnetic separation techniques to isolate particle-free CD25+ cells, and unwanted cells were subsequently targeted for immunomagnetic depletion. These cells are often referred to as regulatory T cells (Tregs) and are used in immune response assays. Isolated Tregs have been cryopreserved in animal component-free CryoStor® CS10 medium.

Cells were obtained using Institutional Review Board (IRB)-approved consent forms and protocols.

Donor Status:	Normal
Characterization Criteria:	Cell count, viability, donor virus testing, age, sex, ethnicity, weight, height
Format:	CD4+CD25+CD127lowFOXP3+ T cells are frozen in CryoStor® CS10.
Purity:	≥ 85% CD3+CD4+CD25+CD127low and ≥ 80% CD3+CD4+CD25+FOXP3+ by flow cytometry
Anticoagulant:	Acid-citrate-dextrose solution A (ACDA)

For donor details, refer to the lot-specific Certificate of Analysis.

Stability and Storage

Product stable at -135°C or colder for 12 months from date of receipt. Short-term storage of cells (< 1 month) at -80°C is acceptable, but should be minimized to ensure maximum stability. Thawed samples must be used immediately. As these are primary cells, they have a finite life span in culture.

Precautions

Donor Screening: Donors are screened for HIV-1, HIV-2, hepatitis B, and hepatitis C.

If the donor has been screened within 90 days of donation the product will be shipped with negative test results from donor screening.

If the donor has not been screened within 90 days of collection, a test sample will be taken at the time of donation and the product will be shipped before the screening results are available. In the unlikely event that a test result is positive, the customer will be contacted as soon as possible (usually within 24 - 72 hours from the time of shipment).

Donors have been tested and found to be negative for HIV-1, HIV-2, hepatitis B, and hepatitis C prior to donation. As testing cannot completely guarantee that the donor was virus-free, THIS PRODUCT SHOULD BE TREATED AS POTENTIALLY INFECTIOUS and only used following appropriate handling precautions such as those described in biological safety level 2. When handling this product, do not use sharps such as needles and syringes.

STEMCELL cannot guarantee the biological function or any other properties associated with performance of cells in a researcher's individual assay or culture systems. STEMCELL assures the cells will meet the specifications only when assessed, before washing, by our test methods.

FOR IN VITRO RESEARCH USE ONLY. NOT APPROVED FOR DIAGNOSTIC, THERAPEUTIC, OR CLINICAL APPLICATIONS. NOT APPROVED FOR HUMAN OR VETERINARY USE IN VIVO.

Directions for Use

IMPORTANT: To confirm the number of cells provided, a viable cell count must be done immediately after thawing (before washing). Work quickly once the cells have been thawed to ensure high viability and recovery. Use sterile technique when processing thawed cells.

1. Warm medium in a 37°C water bath. See Accessory Products (below) for recommended media.
2. Wipe the outside of the vial of cells with 70% ethanol or isopropanol.
3. In a biosafety hood, twist the cap a quarter-turn to relieve internal pressure and then retighten.
4. Quickly thaw cells in a 37°C water bath by gently shaking the vial. Remove the vial when a small frozen cell pellet remains. Do not vortex cells.
NOTE: It is important to work quickly in the following steps to ensure high cell viability and recovery.
5. Wipe the outside of the vial with 70% ethanol or isopropanol.
6. Measure the total volume of the cell suspension using a 2 mL serological pipette. This value is used in step 12 to calculate the number of cells provided.
7. Remove a 20 µL aliquot of cells for counting. If using Trypan Blue to assess viability, for $\geq 1 \times 10^6$ cells we suggest adding a minimum of 20 µL of medium and recording the volume of medium added. For $< 1 \times 10^6$ cells, dilute directly in 20 µL Trypan Blue. Set diluted aliquot aside until step 12. See Notes and Tips section for more details on performing cell counts with a hemocytometer.
8. Transfer the remaining cell suspension to a 50 mL conical tube.
9. Rinse the vial with 1 mL of medium and add it dropwise to the cells, while gently swirling the 50 mL tube.
10. Wash by adding 15 - 20 mL of medium dropwise, while gently swirling the tube.
11. Centrifuge the cell suspension at 300 x g for 10 minutes at room temperature (15 - 25°C).
12. If using Trypan Blue, perform a cell count on the diluted aliquot from step 7.
13. Carefully remove the supernatant (from step 11) with a pipette, leaving a small amount of medium to ensure the cell pellet is not disturbed. Resuspend the cell pellet by gently flicking the tube.
14. If cells are starting to clump, add 100 µg DNase I Solution per mL of cell suspension and incubate for 15 minutes at room temperature.
NOTE: Do not add DNase I Solution if the cells will be used for DNA or RNA extraction.
15. Gently add 15 - 20 mL of medium to the tube.
16. Centrifuge the cell suspension at 300 x g for 10 minutes at room temperature.
17. Carefully remove the supernatant with a pipette, leaving a small amount of medium to ensure cell pellet is not disturbed. Resuspend the cell pellet by gently flicking the tube.
NOTE: Cell loss of up to 30% can be expected during the wash steps.
18. Cells are now ready for use in downstream applications.

Notes and Tips

For a protocol on performing total nucleated cell counts using a hemocytometer, refer to <https://www.stemcell.com/how-to-count-cells-with-a-hemocytometer>.

Accessory Products

PRODUCT NAME	CATALOG #
DNase I Solution (1 mg/mL)	07900
Falcon® Conical Tubes, 50 mL	38010
Falcon® Serological Pipettes, 2 mL	38002
Hausser Scientific™ Bright-Line Hemocytometer	100-1181
Iscove's Modified Dulbecco's Medium (add 10% fetal bovine serum)	36150
StemSpan™ SFEM II	09655
Trypan Blue	07050

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