Primary Cells

Custom Human Primary Cells, Frozen

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Product Description

Custom cell products are produced to meet your unique requirements and were collected using consent forms and protocols approved by either the Food and Drug Administration (FDA), an Institutional Review Board (IRB), the US Department of Health and Human Services, a Research Ethics Committee (REC)-approved consent forms and protocols, and/or an equivalent regulatory authority.

Donor Status:	Normal
Characterization Criteria:	As per custom request
Format:	As per custom request.
Anticoagulant:	As per custom request.

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 lifespan in culture.

Precautions

Peripheral Blood and Bone Marrow Donor Screening: Donors are screened for HIV-1 and -2, hepatitis B, and hepatitis C. Cryopreserved products are shipped with negative test results from donor screening that is performed within 90 days of collection.

Cord Blood Donor Screening: Cord blood is only collected from mothers that have tested negative for HIV-1 and -2, and hepatitis B during their pregnancy. Hepatitis C is tested for at the time of collection. Cryopreserved products are shipped with negative test results from donor screening.

All Products: Donors have been tested and found to be negative for HIV-1 and 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.

Storage of frozen cell products in the vapor phase of a liquid nitrogen storage tank is recommended. Storage in the liquid phase can result in cross-contamination if the vial breaks or is not sealed properly. Storage in the liquid phase also increases the potential for liquid nitrogen to penetrate the vial and cause it to explode when removed from storage. Use of a face shield is required as a safety precaution when transferring cells from one container to another. 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 immediately after thawing (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.

The following instructions are for thawing cells. Instead of using a water bath (steps 1 - 4), cells can be thawed using ThawSTAR® CFT2 Automated Thawing System (Catalog #100-0650). For complete instructions, refer to the Product Information Sheet (Document #10000010334), available at www.stemcell.com, or contact us to request a copy.

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. In a biosafety hood, 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 the cell suspension for counting. To assess viability using Trypan Blue, dilute as follows:
   - For ≥ 1 x 10^6 cells, add ≥ 20 µL of medium and record the volume of medium added.
   - For < 1 x 10^6 cells, dilute directly in 20 µL of Trypan Blue. Set diluted aliquot aside until step 12.

   NOTE: See Notes and Tips 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 of DNase I Solution per mL of cell suspension and incubate at room temperature for 15 minutes.

    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 #
3% Acetic Acid with Methylene Blue	07060
DMEM with 4500 mg/L D-Glucose (add 10% fetal bovine serum)	36250
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
RPMI 1640 Medium (add 10% fetal bovine serum)	36750
ThawSTAR® CFT2 Automated Thawing System	100-0650
Trypan Blue	07050