Primary Cells

Human Bone Marrow Stromal Cells, Frozen

Catalog # 70022 0.75 million cells

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Product Description

Primary human stromal cells (mesenchymal stem cells) were derived from bone marrow (BM) mononuclear cells (MNCs). Stromal cells were culture-expanded in MesenCult™ Proliferation Kit (Human; Catalog #05411) and frozen after the first culture passage.

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

Donor Status:	Normal
Characterization Criteria:	Cell count, viability, purity, donor virus testing, age, sex, ethnicity, weight, height, smoking status, other information
Format:	Stromal cells are frozen in CryoStor™CS10.
Purity:	The purity of stromal cells is ≥ 90% for CD73, CD90, CD105 and < 5% for CD14, CD34, CD45 by flow cytometry.
Anticoagulant:	Heparin

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 and 2, Hepatitis B, and Hepatitis C.

Cryopreserved products are shipped with negative test results from donor screening that is done within 90 days of collection.

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.

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Handling / 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 techniques when processing thawed cells.

1. Warm medium in a 37°C water bath. See Accessory Products (below) for recommended wash 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 viabilty, for ≥ 1 x 10e6 cells we suggest adding a minimum of 20 µL of medium and recording the volume of medium added. For < 1 x 10e6 cells dilute cells directly in 20 µL Trypan Blue. Set diluted aliquot aside until step 12. See Tips section for more details on performing cell counts with a hemacytometer.
8. Transfer the remaining cell suspension to a 50 mL conical tube.
9. Rinse the vial with 1 mL of medium and add it drop-wise to the cells, while gently swirling the 50 mL tube.
10. Wash by adding 15 - 20 mL of medium drop-wise, 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, dilute the aliquot from step 7 (if not previously done) during centrifugation, and count.
13. Carefully remove the supernatant 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 per mL of cell suspension and incubate for 15 minutes at room temperature (15 - 25°C).
    NOTE: Do not add DNase I 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 (15 - 25°C).
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.

Tips

Manual cell counting protocol: http://www.humanimmunologyportal.com/protocols/performing-cell-counts-with-a-hemacytometer/
Converting g to rpm: http://www.humanimmunologyportal.com/hiptools/apps/

Accessory Products

PRODUCT NAME	CATALOG #
Iscove's Modified Dulbecco's Medium (add 10% fetal bovine serum)	36150
DMEM with 1000 mg/L D-glucose (add 10% fetal bovine serum)	36253
RPMI 1640 Medium (add 10% fetal bovine serum)	36750
Trypan Blue	07050
DNase I Solution (1 mg/mL)	07900

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