stem express®
SUSTAINING QUALITY OF LIFE THROUGH RESEARCH™

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Cryopreserved Product

Bone Marrow CD133+ Stem/Progenitor Cells

Catalog #	BM0600C	0.5 million cells
	BM0601C	1 million cells
	BM0602C	2 million cells
	BM0603C	5 million cells

Product Description

Bone Marrow CD133+ Stem/Progenitor Cells (BM-CD133) are positively selected using immunomagnetic anti-CD133 microbeads from bone marrow mononuclear cells.

Cryopreservation

Cryopreserved products allow for prolonged storage before use. Cell products contain 10% DMSO to minimize cell death during freezing. All cryopreserved products are stored in containers designed and tested for ultra-low temperatures at long time intervals. We normally ship cryopreserved items on dry ice, but can also use a cryoshipper at the customer's request.

Sample Collection and Processing

All samples are collected at nearby partner hospitals or clinics. Samples are then quickly processed in our on-site laboratory to achieve maximum viability and quality. Cryopreserved cells are frozen at -1°C / minute in a -80°C freezer, and then transferred to liquid nitrogen.

Format

Isolated cells are normally frozen in CryoStor CS10 (10% DMSO), while we freeze stem / progenitor cells in StemSpan + 10% DMSO. We can also use freezing media as specified by the customer.

Storage

Cryopreserved cells should be maintained at -135°C or colder (in liquid nitrogen). The cells are warrantied for 6 months from the date of receipt if stored at -135°C or colder. Storage of cells at -80°C for less than one month should maintain cell viability but is not covered by the warranty.

Thawing Instructions for Cell Products

Materials

- Frozen Cryovial containing cells
- Desired medium warmed to 37°C
- Sterile 50 mL centrifuge tube
- Water bath warmed to 37°C
- 70% ethanol
- DNase I

Protocol

1. Place the vial into the 37°C water bath. You may consider leaving the vial in a sealed plastic bag to reduce the chance of contamination. Perform this step immediately after removing the Cryovial from the dry ice in the shipment or after removing the Cryovial from your liquid nitrogen storage.
2. Quickly thaw the cells in less than a minute by gently swirling the vial in the water bath until only a small bit of ice remains. Do NOT vortex the cells at any point while thawing, and work quickly to maximize cell viability.
3. Wipe the vial with 70% ethanol and transfer it to a laminar flow hood.
4. Gently mix the cells by inverting the Cryovial. Measure the cell suspension volume in the Cryovial.
5. Aliquot 10 µL of cell suspension from the Cryovial using aseptic technique; have a separate person proceed with the cell count and viability measurement using the Counting Instructions provided.

   Important: This cell viability/counting step is required to ensure the quantity of cells provided. Thawing and counting must be completed simultaneously with two people to ensure accurate measurements in the cell count and to maintain product viability. Be sure to count the cells at this step, before washing, because you will lose cells in the wash.
6. Transfer the cell suspension to the sterile centrifuge tube containing DNase I, which will prevent cell clumping. For mononuclear cells, use 300 µg/mL cell suspension. For purified cell products, use 100 µg/mL cell suspension. DNase I is not necessary if cells are to be lysed for protein or DNA/RNA extraction.
7. Rinse the remaining cells from the Cryovial with 1 mL warmed medium. Slowly add this suspension to the centrifuge tube with the cells in a dropwise fashion, 1 drop every 5 seconds, while gently swirling the tube.
8. Add 15-20 mL warmed medium to the cells. Gently mix by inverting the closed tube.
9. Wash the cells by centrifuging at room temperature, 300 rcf for 10 minutes, with low brake.
10. Check the supernatant for clarity, and check the bottom of the tube for a complete cell pellet. Carefully remove the supernatant with a pipette, leaving a small volume so as to not disturb the pellet. Resuspend the cell pellet by gently flicking the sides of the tube.
11. Again add 15-20 mL warmed medium and perform the wash step once more.
12. Gently resuspend the cells in warmed medium. They are now ready for use.

    Important: Be aware that cell loss is expected and may be up to 30% during thaw and wash steps. Recovery rates vary depending on technique.

Counting Instructions

Materials

- Cleaned hemocytometer
- Trypan Blue
- PBS or other medium

Protocol

1. Dilute the 10 µL of cell suspension with 90 µL of PBS to make a 1-in-10 dilution.Make a further 1-in-2 dilution with equal volumes of diluted cell suspension and Trypan Blue (for a total dilution of 1-in-20).
2. Load one side of the hemocytometer, being careful not to over- or under-fill the chamber.
3. Count viable (clear, round) and non-viable (blue, irregular shape) cells in the four corner squares. Adjust your dilution if there are more than 100 cells / square.
4. Determine the number of total viable cells in the original Cryovial. One square is equal to 100 nL.

   Viability = live cells / all cells
   Cell Concentration = Mean cells/square × Dilution Factor × 104
   Total Cell Count = Cell Concentration × Starting Volume
   Total Viable Cell Count = Total Cell Count × Viability

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Warning

This product contains human tissue or other biological material and MUST be handled at Biosafety Level 2 or higher. All biological products should be treated as potentially infectious or contaminated material, even if infectious disease screening reports are negative. Follow universal precautions and wear appropriate personal protective equipment.

Product Warranty

StemExpress warranties its fresh products if tested immediately upon receipt and if counted exactly as in the above instructions. The cells are guaranteed to meet specifications for viability, purity, and cell count, also provided the above instructions are followed exactly. StemExpress is not able to guarantee cell performance for any in vitro or in vivo culture system, proliferation assay, functional assay, or implantation.

THE PURITY, VIABILITY & QUALITY YOU NEED™