



Human Umbilical Cord Blood CD34⁺ /133⁺ Progenitor Cell Care Manual

INSTRUCTION MANUAL ZBM0065.04

SHIPPING CONDITIONS

- **Human Umbilical Cord Blood CD34⁺/133⁺ Progenitor Cells, cryopreserved**

Cryopreserved human umbilical cord blood CD34⁺ progenitor cells are shipped on dry ice and should be stored in liquid nitrogen vapor phase immediately upon arrival. Orders are delivered via overnight courier. **Must be processed immediately upon shipment receipt.**

STORAGE CONDITIONS

- **Cryopreserved cells:** Vials of frozen HUCB CD34⁺/133⁺ progenitor cells are to be stored in vapor phase nitrogen (-150°C to -190°C).
- **Lymphocyte Medium:** Store 2-8°C.

All Zen-Bio Inc products are for research use only. Not approved for human or veterinary use or for use in diagnostic or clinical procedures.

ORDERING INFORMATION AND TECHNICAL SERVICES

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LIMITED PRODUCT WARRANTY

This warranty limits our liability to replacement of this product. No other warranties of any kind, expressed or implied, including without limitation implied warranties of merchantability or fitness for a particular purpose, are provided by Zen-Bio, Inc. Zen-Bio, Inc. shall have no liability for any direct, indirect, consequential, or incidental damages arising out of the use, the results of use, or the inability to use this product.

Zen-Bio, Inc warrants its cells only if Zen-Bio media are used and the recommended protocols are followed. Cryopreserved human blood cells are assured to be viable when thawed according to Zen-Bio protocols.

Contact ZenBio, Inc. within no more than 24 hours after receipt of products for all claims regarding shipment damage, incorrect ordering or other delivery issues. Delivery claims received after 7 days of receipt of products are not subject to replacement or refund.

PRECAUTIONS

This product is for research use only. It is not intended for human, veterinary, or in vitro diagnostic use. Proper precautions and biological containment should be taken when handling cells of human origin, due to their potential biohazardous nature. Always wear gloves and work behind a protective screen when handling primary human cells. All media, supplements, and tissue culture ware used in this protocol should be sterile.

To comply with U.S. Food and Drug Administration (FDA) regulations, these products are not for use in Clinical Diagnostic or Therapeutic Procedures.

By your acceptance of these products, you are acknowledging that these products will be:

1. Treated as potentially contaminated biological specimens even if accompanying serological reports are negative;
2. Handled by establishing or following appropriate safety control procedures to ensure the safety of using these products.

INTRODUCTION

CD34 is a well-known cell surface marker for primitive and bone marrow-derived progenitor cells, especially for hematopoietic and endothelial progenitors. Within cord blood there are CD34+ and another population that is both CD34+/CD133+. Zen-Bio offers CD34+/CD133+ progenitor cells in a phenotypically undifferentiated state. We have found the lots that exhibit high percentage CD34+/CD133+ cells perform much better in differentiation. The progenitor cells are derived from cord blood of consented healthy donors and produced at the Zen-Bio facility. CD34+ progenitor cells are suitable for a series of studies for directed differentiation into more committed types of blood cells and endothelial lineages. Immediately after isolation, the freshly prepared CD34+/CD133+ progenitor cells are cryopreserved using a serum-free cryopreservation medium. Each vial contains $\geq 100,000$ cells per vial.

QUALITY CONTROL

Quality control tests are performed for each lot of CD34+/133+ progenitor cells. They are characterized by flow cytometry analyzing viability, cell size, and the presence of CD34, CD45, CD133, and CD31 markers. Cells have a guaranteed purity of >90% and a viability >80%. In addition, all blood products have been tested for some common blood borne pathogens and microbial contaminants.

MATERIALS PROVIDED FOR EACH CATALOG ITEM

❖ Cryopreserved Human Umbilical Cord Blood CD34+/CD133+ progenitor cells

-Catalog # SER-CD34-F

Frozen vial containing $\geq 100,000$ cells/vial of Human Umbilical Cord Blood (HUCB) CD34+/133+ Progenitor cells

LYMPHOCYTE MEDIUM COMPOSITION

Recommended product.

Cat# LYMPH-1 (100ml); LYMPH-1-50 (50ml)

RPMI 1640

L-Glutamine

Fetal Bovine Serum

DNase I

Penicillin

Streptomycin

Amphotericin B

THAWING CRYOPRESERVED HUCB CD34⁺/CD133⁺ PROGENITOR CELLS

1. Warm Lymphocyte Medium (cat# LYMPH-1) to 37°C.
2. Rapidly thaw the vial of frozen cells in a 37°C water bath until just prior to complete thawing (slurry of residual ice should be present). Wipe the outside of the vial with 70% ethanol.
3. Aseptically transfer the cell suspension to a 50mL conical tube.
4. Rinse the vial with 1 mL of medium. Then slowly add drop wise to the cells in the 50 mL conical tube while gently swirling the tube.
5. Slowly add medium drop wise to the 50 mL tube until the total volume reaches 25 mL.
6. Centrifuge the cell suspension at 400x g at room temperature for 10 minutes.
7. Carefully remove the supernatant and save in a second tube leaving 1 mL behind as not to disturb the pellet. Gently resuspend the cells up to a volume of 2 mL (2 mL per vial of product). Count the number of cells. If count is lower than expected, centrifuge the wash that was saved at a higher speed, count and combine if necessary.
8. Gently resuspend cells to desired concentration in media suitable for your application. Please note the Lymphocyte Medium is not suitable as a culture medium.

FREQUENTLY ASKED QUESTIONS

1. **Must I use your Lymphocyte Medium?** Yes, we strongly recommend the use of our Lymphocyte Medium to thaw the cells as it will prevent clumping and maximize viability upon thawing. If you are using a homemade formulation and not achieving success, please use our Lymphocyte Medium in a variety of convenient sizes to suit your needs (catalog # LYMPH-1, LYMPH-1-50).
2. **Can I use your Lymphocyte Medium to culture my cells?** No. Our Lymphocyte Medium is NOT a culture or a growth medium. It is a medium designed to successfully thaw blood derived cells with high viability and less clumping of the subpopulations of cells that remain in suspension.

3. **Do you test for pathogens? Which ones?** Yes. Samples from each donor are tested via PCR to confirm non-reactivity for HIV-1, HIV-2, syphilis, CMV, hepatitis B and hepatitis C. However, since we cannot test all pathogens, please treat the culture as a potentially infectious agent at Biosafety Level 2 or higher.
4. **What donor information do I receive?** The donor's age, race, and gender are provided in the certificate of analysis that accompanies each lot of cells.
5. **Do you have any protocols for ways to use the cells?** No. We do not provide any protocols for the use of the cells.
6. **My cells have low viability and are clumping upon thawing. Is there a problem with my cells?** We first eliminate any shipping or storage issues as a potential source of your issues. All our cells are quality tested with a minimum viability greater than 80% upon thawing from cryopreservation. We strongly suggest the use of our Lymphocyte Medium to thaw the cells as it will prevent clumping and maximize viability upon thawing. If you are using a homemade formulation and not achieving success, please use our Lymphocyte Medium (catalog # LYMPH-1, LYMPH-1-50).
7. **My cells are not attaching or proliferating. What is wrong?** Nothing is wrong. We recommend that you thaw and use the cells directly. The factors used to treat your cells will depend on your research goal. Our Lymphocyte Medium is NOT a culture or growth medium but a medium designed to successfully thaw blood derived cells.

PATHOGEN TESTING

Samples from each donor are tested via PCR to confirm non-reactivity for HIV-1, HIV-2, syphilis, CMV, hepatitis B and hepatitis C. However, no known test can offer complete assurance that the cells are pathogen free. Our products are tested and are free from mycoplasma contamination. Proper precautions and biological containment should be taken when handling cells of human origin, due to their potential biohazardous nature. All human based products should be handled at a BSL-2 (Biosafety Level 2) or higher. Always wear gloves and work behind a protective screen when handling primary human cells.

REFERENCE

Ngoma, A et al CD34+ Cell Enumeration by Flow Cytometry. 2011 *Arch Pathol Lab Med* 135:909-914.