Human Bone Marrow CD34+ Cell Care Manual

INSTRUCTION MANUAL ZBM0104.01

SHIPPING CONDITIONS

Human Bone Marrow CD34+ Cells, cryopreserved

All US and Canada orders are shipped via Federal Express Priority service and are usually received the next day. International orders are shipped using dry ice or using a dry vapor shipper if transit time will exceed 3 days. Primary human cells are sensitive to extended times (>3 days) transported using dry ice. Please inquire for dry vapor shipper availability if your total transit time will exceed 3 days. Cells should always be stored in liquid nitrogen vapor phase immediately upon arrival. Must be processed immediately upon shipment receipt.

STORAGE CONDITIONS

Cryopreserved cells: Cryopreserved Human Bone Marrow CD34+ cells are to be stored in vapor phase nitrogen (-150°C to -190°C) immediately upon arrival

Lymphocyte Medium: Short Term (30 days from ship date) **4°C** 6 months **-20°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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THIS MANUAL IS SUITABLE FOR USE WITH THE FOLLOWING PRODUCTS:

SER-BMCD34-F	Cryopreserved Human Bone Marrow CD34+ cells, 500,000 cells/vial

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, recommended protocols and storage conditions are followed without amendment or substitution.

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, therapeutic, 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

The progenitor cells are derived from the bone marrow of consented ZenBio human bone marrow CD34+ cells are isolated from bone marrow from consented adult donors in the United States. Each sample is derived from a competent volunteer adult donor who has signed an Institutional Review Board (IRB) or US Food and Drug Administration (FDA) validated donor consent form that specifically lists both the intended uses for the donation for non-clinical research and confirms the procedures for processing the samples are Standard Operating Procedure (SOP) managed GLP protocols in compliance with ethical regulations. All samples are collected and processed in the United States.

CD34 is a well-known cell surface marker for primitive and bone marrow-derived progenitor cells, especially for hematopoietic and endothelial progenitors. Within the bone marrow there are CD34+ progenitor cells that exist in a phenotypically undifferentiated state. 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+ progenitor cells are cryopreserved using a serum-free cryopreservation medium. Each vial contains 500,000 viable cells per vial.

PATHOGEN TESTING

Each lot is tested via PCR and found non-reactive to viral DNA from Hepatitis B and viral RNA from HIV 1, HIV-2 and Hepatitis C. Hepatitis B Surface antigen (HBsAg) and HIV antibody (Ab), and STS (Syphilis) are also found non-reactive by US Food and Drug Administration (FDA) licensed tests. No known test can offer complete assurance that these viruses are not present. Since we cannot test all pathogens, always treat the culture as a potentially infectious reagent. We recommend using the US Centers for Disease Control (CDC) Universal Precautions for prevention of blood-borne pathogens as a minimum guideline for standards of practice at Biosafety Level 1 or higher.

QUALITY CONTROL:

Human bone marrow CD34+ cells are assessed for viability (>70%) and cell surface marker analysis for CD34 (>80%) assessed via flow cytometry.

MATERIALS PROVIDED FOR EACH CATALOG ITEM

Cryopreserved Normal Human Bone Marrow CD34+ cells, 500,000 cells/vial

- Catalog # SER-BMCD34-F
- STORE VAPOR PHASE LIQUID NITROGEN IMMEDIETELY UPON ARRIVAL.

LYMPHOCYTE MEDIUM COMPOSITION

Recommended product.

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

RPMI 1640
L-Glutamine
Fetal Bovine Serum (FBS; US Origin)
DNAse I
Penicillin
Streptomycin
Amphotericin B

THAWING CRYOPRESERVED Human Bone Marrow $CD34^+$ CELLS

1. Use only sterile tissue culture treated cultureware in a sterile environment.
2. Warm Lymphocyte Medium (cat# LYMPH-1) to 37°C.
3. 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.
4. Aseptically transfer the cell suspension to a 50mL conical tube.
5. Rinse the vial with 1 mL of Lymphocyte Medium. Then slowly add drop wise to the cells in the 50 mL conical tube while gently swirling the tube.
6. Slowly add medium drop wise to the 50 mL tube until the total volume reaches 25 mL.
7. Centrifuge the cell suspension at 400x g at room temperature for 10 minutes.
8. 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.
9. 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, Hepatitis B and Hepatitis C. Since we cannot test all pathogens, always treat the culture as a potentially infectious reagent. We recommend using the US Centers for Disease Control (CDC) Universal Precautions for prevention of blood-borne pathogens as a minimum guideline for standards of practice at Biosafety Level 1 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?**
    a. We first eliminate any shipping or storage issues as a potential source of your issues. All cells should arrive in frozen condition and be stored in vapor phase liquid nitrogen immediately upon arrival.
    b. Our cells are quality tested with a minimum viability greater than 70% upon thawing. 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.

REFERENCE

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