Human CD34+ Cell Care Manual

INSTRUCTION MANUAL ZBM0065.08

SHIPPING CONDITIONS

Orders are delivered via Federal Express courier. All USA and Canada orders are shipped via Federal Express Priority service and are usually received the next day. Non North American International orders are usually received in 2-4 days. Primary human cells can be sensitive to extended times at dry ice temperatures. If your transit time will exceed 3 days, please inquire about dry vapor shipper options. Please inquire if alternate couriers are needed.

All orders should be processed immediately upon shipment receipt.

STORAGE CONDITIONS

Media: +4°C Expires 30 days from ship date.
-20°C Expires 6 months from ship date.

Cells: Store in vapor phase nitrogen (-150°C to -190°C) IMMEDIATELY UPON RECEIPT.

Any other use negates the warranty.

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

ORDERING INFORMATION AND TECHNICAL SERVICES

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THIS MANUAL IS SUITABLE FOR USE WITH THE FOLLOWING CELL PRODUCTS:

SER-CD34-F	NORMAL HUMAN CORD BLOOD CD34+/CD133+ CELLS, CRYOPRESERVED, 100,000 CELLS/VIAL
SER-CD34-1F	NORMAL HUMAN CORD BLOOD CD34+/CD133+ CELLS, CRYOPRESERVED, SINGLE DONOR, 1 MILLION CELLS/VIAL
SER-CD34-MPB-F	HUMAN MOBILIZED PERIPHERAL BLOOD CD34+ CELLS, CRYOPRESERVED, 5 MILLION CELLS/VIAL
SER-CD34-MPB1-F	HUMAN MOBILIZED PERIPHERAL BLOOD CD34+ CELLS, CRYOPRESERVED, 1 MILLION CELLS/VIAL
SER-BMCD34-F	NORMAL HUMAN BONE MARROW CD34+ CELLS, CRYOPRESERVED, SINGLE DONOR, 500,000 CELLS/VIAL

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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 the performance of cells only if Zen-Bio media are used and the recommended storage conditions and protocols are followed without amendment or substitution. ZenBio, Inc. cryopreserved cells are assured to be viable when stored as recommended and thawed according to Zen-Bio protocols and using the recommended protocol.

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 cultureware used in this protocol should be sterile.

Human CD34+ cell viability depends greatly on the use of suitable media, reagents, and sterile tissue culture treated plastic ware. If these parameters are not carefully observed, cell growth may be slower than expected.

INTRODUCTION

All Zen-Bio human CD34+ cells are derived from cord blood, peripheral blood, or 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 the intended uses for the donation for non-clinical research and confirms the procedures for processing the samples are Standard Operating Procedure (SOP) managed protocols in compliance with ethical regulations. All samples are collected and processed in the United States. Immediately after isolation, the freshly prepared CD34+ cells are cryopreserved using an animal component-free cryopreservation medium.

CD34 is a well-known cell surface marker for primitive and bone marrow-derived progenitor cells, especially for hematopoietic and endothelial progenitors.

INTRODUCTION: HUMAN UMBILICAL CORD BLOOD CD34+/CD133+ PROGENITOR CELLS

Zen-Bio CD34+/CD133+ progenitor cells are derived from cord blood of consented healthy donors and processed at the Zen-Bio facility. Zen-Bio offers CD34+/CD133+ progenitor cells in a phenotypically undifferentiated state. Within cord blood there are CD34+ cells and another population that is both CD34+/CD133+. We have found the lots that exhibit high percentage CD34+/CD133+ cells perform much better in differentiation. CD34+ progenitor cells are suitable for a series of studies for directed differentiation into more committed types of blood cells and endothelial lineages. Each vial contains either 100,000 (SER-CD34-F) or 1 million (SER-CD34-1F) viable cells per vial.

INTRODUCTION: MOBILIZED PERIPHERAL BLOOD CD34+ CELLS

Zen-Bio mobilized peripheral blood CD34+ cells are derived from consented adult volunteer donors and processed at the Zen-Bio facility. Competent volunteer donors are injected with Granulocyte colony stimulating factor (G-CSF) to stimulate the bone marrow to produce a large number of hematopoietic cells and mobilizes them into the peripheral blood stream. The CD34+ cell subset is derived from the blood of G-CSF stimulated donors and isolated using positive immunomagnetic cell separation methods. Each vial contains either 5 million (SER-CD34-MPB-F) or 1 million (SER-CD34-MPB1-F) viable cells per vial.

INTRODUCTION: NORMAL HUMAN BONE MARROW CD34+ CELLS

Zen-Bio human bone marrow CD34+ cells are isolated from bone marrow from consented adult donors and processed at the Zen-Bio facility. 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. Each vial contains 500,000 viable cells per vial.

QUALITY CONTROL

Quality control tests are performed for each lot of human umbilical cord blood CD34+/133+ derived progenitor cells, catalog number SER-CD34-F (500,000 cells/vial) or catalog number SER-CD34-1F (1 million cells/vial). They are characterized by flow cytometry analyzing viability, cell size, and the presence of CD34 and CD133 cell surface markers.

Quality control tests are performed for each lot of mobilized peripheral blood derived CD34+ cells, catalog number SER-CD34-MPB-F (5 million cells/vial) or catalog number SER-CD34-MPB1-F (1 million cells/vial). They are characterized by flow cytometry analyzing viability, cell size, and the presence of CD34 cell surface marker.

Quality control tests are performed for each lot of human bone marrow CD34+ cells, catalog number (500,000 cells/vial). They are characterized by flow cytometry analyzing viability, cell size, and the presence of CD34 cell surface marker.

All cell types present in this manual have a guaranteed purity of $\geq$80% and a viability of >70%. In addition, all blood products have been tested for some common blood borne pathogens and microbial contaminants. For more information on pathogen testing, read section titled Pathogen Testing.

CATALOG ITEMS

- **Lymphocyte Medium**
  - Cat # LYMPH-1 (100mL), LYMPH-1-50 (50mL)
  - Store according to label
  - THIS MEDIUM IS FOR THAWING BLOOD-DERIVED PRODUCTS ONLY, IT IS NOT A CULTURE MEDIUM

- **Cryopreserved Normal Human Cord Blood CD34/CD133+ Cells**
  - Cat # SER-CD34-F (100,000 cells/vial) Single or Pooled lot
    SER-CD34-1F (1 million cells/vial) Single Donor Lot
  - Cryopreserved vial containing normal human cord blood CD34/CD133+ cells **(store in vapor phase liquid nitrogen IMMEDIATELY upon receipt)** *any other storage negates the warranty*

- **Cryopreserved Human Mobilized Peripheral Blood CD34+ Cells**
  - Cat # SER-CD34-MPB-F (5 million cells/vial)
    SER-CD34-MPB1-F (1 million cells/vial)
  - Cryopreserved vial containing human mobilized peripheral blood CD34+ cells **(store in vapor phase liquid nitrogen IMMEDIATELY upon receipt)** *any other storage negates the warranty*

- **Cryopreserved Human Bone Marrow CD34+ Cells**
  - Cat # SER-CD34-MPB-F (500,000 cells/vial)
  - Cryopreserved vial containing normal human bone marrow CD34+ cells **(store in vapor phase liquid nitrogen IMMEDIATELY upon receipt)** *any other storage negates the warranty*

MEDIUM COMPOSITION

Lymphocyte Medium (Cat# LYMPH-1)	**Storage and Expiration Date**
RPMI-1640, 300 mg/L (2.05 mmol/L) L-glutamine Fetal Bovine Serum (FBS; USA Origin) Deoxyribonuclease I (from bovine pancreas) Penicillin Streptomycin Amphotericin B	• If stored at 4°C upon arrival, the media is stable until the expiration date on the bottle. • If stored at -20°C upon arrival, the media is stable for 6 months. *The media will expire 30 days after the thaw date.* • Medium is provided ready to use and prepared fresh prior to shipment. **This is NOT a culture medium, Lymphocyte Medium is for thawing cryopreserved blood-derived cells ONLY.**

THAWING CRYOPRESERVED CELLS

INSTRUCTIONS APPLICABLE FOR ALL PRODUCTS IN THIS MANUAL

Note: *Primary human cell viability is greatly dependent on the use of appropriate sterile tissue culture treated cultureware. No extracellular matrix coatings required.* **Products listed in this manual are for single thaw and use only.**

1. Pre-warm Lymphocyte Medium (cat# LYMPH-1) at 37°C, and prepare all pipets and vessels.
2. Remove cryovial of CD34+ cells from liquid nitrogen and place **immediately** into a 37°C water bath with mild agitation. Be careful not to submerge the cap of the vial into water. For best results, the thawing step should not take more than 1 minute, and should be stopped when there is still visible ice within the vial.
3. Rinse the outside of the cryovial with 70% ethanol, and wipe the cryovial with lint-free lab wiper before transferring to a biosafety cabinet.
4. Open the cryovial under laminar flow hood and transfer the cell suspension to a 50mL conical tube.
5. Rinse the inside of the empty vial with 1mL LYMPH-1 and transfer it to the same 50mL conical tube. Slowly add LYMPH-1 drop wise to the 50mL conical tube until the total volume reaches 25mL.
6. Centrifuge cell suspension at 400 x *g* for 10 minutes at room temperature.
7. Carefully transfer the supernatant and save in a second 50mL conical tube, leaving 1mL behind so as not to disturb the cell pellet.
8. Gently resuspend the cells up to a volume of 2mL total (or 2mL per vial of product). Count the number of cells using a hemocytometer or automated cell counter.

   Note: If the count is lower than expected, centrifuge the supernatant that was set aside in step 7 at a higher speed. Count the new cell pellet and combine the cell pellets if necessary.
9. Gently resuspend the cells to the appropriate concentration as per your protocol.

FREQUENTLY ASKED QUESTIONS

Must I use your Lymphocyte Medium?

Yes, we strongly recommend the use of our Lymphocyte Medium to thaw blood-derived 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. We sell our Lymphocyte Medium in a variety of convenient sizes to suit your needs (catalog # LYMPH-1, LYMPH-1-50).

Can I use your Lymphocyte Medium to culture my PBMCs?

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. You must provide your own protocols and culture medium for your experiments.

Do you test for pathogens? Which ones?

Yes. Please refer to the section titled Pathogen Testing for more information.

What donor information do I receive?

The donor's age, gender, and race are provided in the certificate of analysis that accompanies each lot of cells.

Do you have any protocols for ways to use the cells?

No. We do not provide any protocols for the use of any of the CD34+ cells. The uses for these products are too varied to provide a comprehensive protocol suitable for each experiment.

My cells have low viability and are clumping upon thawing. Is there a problem with my cells?

We first eliminate any shipping delays or product storage issues as a potential source of your issues. All our cells are quality tested with a minimum viability $\geq$70% 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).

My cells are not attaching or proliferating. What is wrong?

We recommend that you thaw using our recommended protocols and Lymphocyte Medium and use the cells immediately in your experiments. Zen-Bio only provides LYMPH-1 to successfully thaw the CD34+ cells when thawed according to the instructions provided in this manual. You may then use your experimental media for your specific experiments. Zen-Bio, Inc. does not provide protocols for uses of CD34+ cells.

What cryopreservation medium is used for the CD34+ cells?

All ZenBio, Inc CD34+ cells are cryopreserved in serum-free, animal component free cryopreservation medium.

PATHOGEN TESTING

Samples from each donor are tested via PCR to confirm non-reactivity for HIV-1, HIV-2, syphilis, 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-1 (Biosafety Level 1) or higher. Always wear gloves and work behind a protective screen when handling primary human cells.

REFERENCES

- Ngoma,A. et al. (2011) CD34 Cell Enumeration by Flow Cytometry: A Comparison of Systems and Methodologies. Archives of Pathology & Laboratory Medicine: July 2011, Vol. 135, No. 7, pp. 909-914. *Archives of Pathology & Laboratory Medicine 2011 135*:*7*, *909-914*
- *Bender, JG et al "Identification and comparison of CD34-positive cells and their subpopulations from normal peripheral blood and bone marrow* using multicolor flow cytometry." Blood 77.12 (1991): 2591-2596. Web. 14 Sept 2018.