



Human Bone Marrow Mononuclear Cell (MNC) Manual

INSTRUCTION MANUAL ZBM0131.00

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. International orders are usually received in 2-4 days. Unlike cell lines, 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

- ❖ Lymphocyte Medium:
 - Stored +4°C Expires 30 days from ship date
 - Stored -20°C Expires 6 months from ship date
- ❖ Cryopreserved cells: Vials of frozen preadipocytes are to be stored in vapor phase nitrogen (-150°C to -190°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

ZenBio, Inc.

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Durham, NC 27713 U.S.A.

Telephone

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Electronic mail (e-mail)

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THIS MANUAL IS SUITABLE FOR THE FOLLOWING PRODUCT

SER-BMMNC-F	HUMAN BONE MARROW MONONUCLEAR CELLS, 10 MILLION 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 its cells only if Zen-Bio media, recommended shipping conditions, recommended storage conditions and handling protocols are followed. Cryopreserved human cells are assured to be viable when stored and 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:

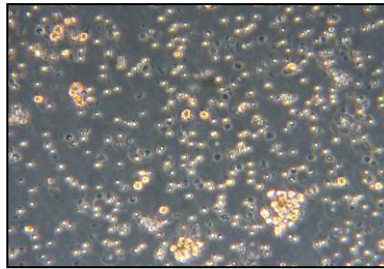
1. Treated as potentially contaminated biological specimens even if accompanying serological reports are negative;
2. Handled by establishing or following appropriate safety control procedures to ensure the safety of using these products.

INTENDED USE

Cryopreserved Human Bone Marrow Mononuclear Cells (MNCs) are available as positive and negative controls for T-cell monitoring in ELISPOT, ELISA, cytokine bead array, tetramer/pentamer, and flow cytometry assays. We do not provide protocols. The cells are for research uses only and not for clinical, diagnostic, therapeutic or other uses in humans.

INTRODUCTION

Mononuclear cells (MNCs) is defined as any blood cell with a round nucleus (i.e. a lymphocyte or a monocyte). These blood cells are a critical component in the immune system to fight infection and adapt to intruders. The lymphocyte population consists of CD4+ and CD8+ T cells, B cells and Natural Killer cells, CD14+ Monocytes, and Basophils/Neutrophils/Eosinophils/Dendritic cells. These cells are often extracted from bone marrow aspirate using Ficoll, a hydrophilic polysaccharide that separates layers of blood, with monocytes and lymphocytes forming a buffy coat. This buffy coat contains the MNCs. Additionally; MNCs can be extracted from bone marrow using a hypotonic lysis which will preferentially lyse red blood cells. This method results in neutrophils and other polymorphonuclear (PMN) cells, which are important in innate immune defense being obtained.



QUALITY CONTROL

Quality control tests are performed for each lot of Human Bone Marrow Mononuclear cells. The cells are assessed for viability and characterized by their surface markers via flow cytometry. Population distributions expressed as percentage positive are presented on the certificate of analysis for each lot of cells. Cells have a guaranteed purity of >90% and a viability a minimum 80%.

Samples from each donor are tested via PCR to confirm non-reactivity for HIV-1, HIV-2, Hepatitis B Hepatitis C and syphilis. 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.



MATERIALS PROVIDED FOR EACH CATALOG ITEM

❖ Cryopreserved human bone marrow mononuclear cells

Catalog # SER-BMMNC-F

Cryopreserved vial containing 10 million viable cells/vial of human bone marrow mononuclear cells

Store in vapor phase liquid nitrogen. Any other storage negates the warranty.

LYMPHOCYTE MEDIUM COMPOSITION

Recommended product.

Catalog Number	Components	Expiration Dates
LYMPH-1 (100ml) LYMPH-1-50 (50ml)	RPMI 1640 L-Glutamine Fetal Bovine Serum (US Origin) DNase I Penicillin Streptomycin Amphotericin B	<p>If placed at 4°C upon arrival, the media is stable until the +4°C expiration date on the bottle label.</p> <p>-If stored at -20°C upon arrival, the media is stable until the -20°C 6 months post ship date listed on the bottle label.</p> <p>-Add fresh antibiotics at 1% when you are ready to use.</p> <p>-The media will expire 30 days after the thaw date. Store at +4°C</p>

THAWING CRYOPRESERVED BONE MARROW MONONUCLEAR CELLS (MNC)

Please note our Lymphocyte Medium is NOT a culture or a growth medium. It is a medium designed to successfully thaw blood/bone marrow derived cells with high viability

1. Warm Lymphocyte Medium (Cat # LYMPH-1 or LYMPH-1-50) to 37°C.
2. Rapidly thaw the vial of cryopreserved 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.
8. Gently resuspend the cells up to a volume of 2 mL (2 mL per vial of product). Count the number of cells. If viability is lower than expected, re-spin at 100xg for 10 minutes and recount.
9. Gently resuspend cells to desired concentration as per your protocol.

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 MNCs?** 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, Cytomegalovirus (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 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 blood mononuclear cells. The uses for this product are too varied to provide a comprehensive protocol suitable for each experiment.
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 of 80% upon thawing from cryopreservation. Once shipping or storage issues have been eliminated, try re-spinning the cells at 100xg and then recounting. We also 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 in order to maintain the normal ratio of cells contained in the MNC fraction. The MNC contains a mixed population of cells including lymphocytes and monocytes that will perform in culture in different ways. 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, Hepatitis B, Hepatitis C and syphilis. 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.