Human Peripheral Blood Mononuclear Cell (PBMC) Manual

INSTRUCTION MANUAL ZBM0063.05

SHIPPING CONDITIONS

- **Human Peripheral Blood Mononuclear Cells, cryopreserved**

Cryopreserved human peripheral blood mononuclear 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.**

- **Human Peripheral Blood Mononuclear Cells, Fresh**
- Fresh human peripheral blood mononuclear cells are shipped on cold packs via overnight courier and can be stored at 4°C or used immediately for your studies. **Must be processed immediately upon shipment receipt.**

STORAGE CONDITIONS

- **Cryopreserved cells:** Vials of frozen PBMCs are to be stored in vapor phase nitrogen (-150°C to -190°C).
- **Fresh Cells**: Vials of freshly isolated PBMCs are to be stored at 2-8°C or used immediately for your studies.
- **Lymphocyte Medium**: Media: Short Term 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.

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:

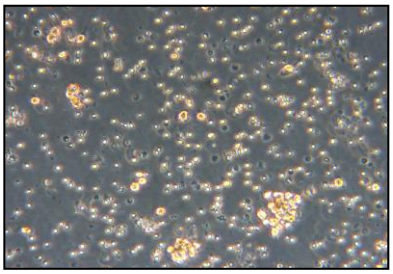
1. Treated as potentially contaminated biological specimens even if accompanying serological reports are negative;
2. Handled by establishing or following appropriate safety control procedures to ensure the safety of using these products.

INTENDED USE

Cryopreserved Human Peripheral Blood Mononuclear Cells (PBMCs) 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.

INTRODUCTION

A peripheral blood mononuclear cell (PBMC) 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 whole blood or from Leukopak using Ficoll, a hydrophilic polysaccharide that separates layers of blood, with monocytes and lymphocytes forming a buffy coat under a layer of plasma. This buffy coat contains the PBMCs. Additionally; PBMCs can be extracted from whole blood 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 Peripheral Blood Mononuclear cells. The cells are 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 >95% and a viability >80%. In addition, all blood products have been tested for common blood borne pathogens and microbial contaminants.

MATERIALS PROVIDED FOR EACH CATALOG ITEM

- **Cryopreserved human peripheral blood mononuclear cells**

  -Catalog # SER-PBMC-F

  Frozen vial containing ≥15 million cells/vial of human peripheral blood mononuclear cells

  -Catalog # SER-PBMC-200-F

  Frozen vial containing ≥100 million cells/vial of human peripheral blood mononuclear cells

- **Fresh human peripheral blood mononuclear cells**

  -Catalog # SER-PBMC

  Vial containing ≥15 million cells/vial of freshly isolated human peripheral blood mononuclear cells

  -Catalog # SER-PBMC-200

  Vial containing ≥ 200 million cells/vial of freshly isolated human peripheral blood mononuclear 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 PERIPHERAL BLOOD MONONUCLEAR CELLS (PBMC)

1. Warm Lymphocyte Medium (Cat # LYMPH-1 or LYMPH-1-50) 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.
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 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.

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, HTLV I, HTLV II, 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 peripheral 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 greater than 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 PBMC fraction. The PBMC 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.

8. **I received fresh PBMC and stored them in refrigerator upon receipt. How long are the cells viable?** Freshly isolated PBMC that have been received may be stored refrigerated upon arrival for 3-5 days; this is highly variable depending on the donor. We do not have any data on cell viability or any changes in the ratios of the cell types beyond this time. It is recommended that you coordinate your order arrival to match your experimental timeline. We recommend using the cells upon receipt to maintain the highest viability.

PATHOGEN TESTING

Samples from each donor are tested via PCR to confirm non-reactivity for HIV-1, HIV-2, HTLV I, HTLV II, 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.