Human Peripheral Blood Mononuclear Cell (PBMC) Care Manual

INSTRUCTION MANUAL ZBM0063.06

SHIPPING CONDITIONS

Human Peripheral Blood Mononuclear Cells, Cryopreserved

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. If your transit time for cryopreserved cells will exceed 3 days, please inquire about dry vapor shipper options. Please inquire if alternate couriers are needed.

Human Peripheral Blood Mononuclear Cells, Fresh

Fresh Human PBMCs are delivered to the USA and Canada ONLY. Fresh primary cells are shipped on cold packs via overnight courier and can be stored at +2-8°C or used immediately for your studies. If stored at +2-8° the cells MUST be used NO LATER THAN 2-3 hours after receipt for optimal viability.

All orders should be processed immediately upon shipment receipt.

STORAGE CONDITIONS

Lymphocyte Media:	+4°C	Expires 30 days from ship date.
	-20°C	Expires 6 months from ship date.
Fresh Cells:	**Process or store at +2-8°C immediately upon receipt.** Store no more than 2-3 **hours** for best results, can be stored 1-2 days maximum.	
Cryopreserved 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

ZenBio, Inc.
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Durham, NC 27713 U.S.A.

Telephone	+1 (919) 547-0692
Toll free (continental US only)	1-866-ADIPOSE 1-(866)-234-7673
Electronic mail (e-mail)	information@zen-bio.com
World Wide Web	http://www.zen-bio.com

THIS MANUAL IS SUITABLE FOR USE WITH THE FOLLOWING PRODUCTS:

SER-PBMC; SER-PBMC-200	HUMAN PERIPHERAL BLOOD MONONUCLEAR CELLS (PBMCS), FRESH
SER-PBMC-F; SER-PBMC-200-F	HUMAN PERIPHERAL BLOOD MONONUCLEAR CELLS (PBMCS), CRYOPRESERVED
SER-PBMC-P-F; SER-PBMC-200P-F	HUMAN PERIPHERAL BLOOD MONONUCLEAR CELLS (PBMCS), CRYOPRESERVED (POOLED)
SER-D-PBMC	HUMAN PERIPHERAL BLOOD MONONUCLEAR CELLS (PBMCS), TYPE 2 DIABETIC, CRYOPRESERVED

CONTENTS

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

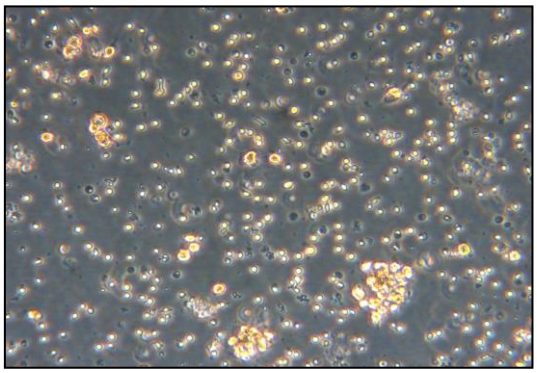
This product is for research use only. It is not intended for human, veterinary, or in vitro diagnostic use. Proper precautions and biological containment should be taken when handling cells of human origin, due to their potential biohazardous nature. **Always wear gloves and work behind a protective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

Human peripheral blood mononuclear cell viability depends greatly on the use of suitable media, reagents, and sterile plastic wear. If these parameters are not carefully observed, cell viability may not be as good as expected.

INTRODUCTION

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 for these procedures.

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 (read Pathogen Testing section for more information).

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

❖ **Fresh Human Peripheral Blood Mononuclear Cells**
- Cat # SER-PBMC (15 million viable cells/vial)
  SER-PBMC-200 (200 million viable cells/vial)
- Fresh vial containing human peripheral blood mononuclear cells **(store at +2-8°C or use IMMEDIATELY upon receipt; use within 2 days)** *any other storage negates the warranty*

❖ **Cryopreserved Human Peripheral Blood Mononuclear Cells**
- Cat # SER-PBMC-F (15 million viable cells/vial)
  SER-PBMC-200-F (100 million viable cells/vial)
  SER-PBMC-P-F (POOLED, 15 million viable cells/vial)
  SER-PBMC-200P-F (POOLED, 100 million viable cells/vial)
  SER-D-PBMC (Type 2 Diabetic, 15 million viable cells/vial)
- Cryopreserved vial containing human peripheral blood mononuclear 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 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 cells ONLY.**

THAWING CRYOPRESERVED CELLS
HUMAN PERIPHERAL BLOOD MONONUCLEAR CELLS

Note: *Primary human cell viability is greatly dependent on the use of appropriate sterile tissue culture treated cultureware. No extracellular matrix coatings required.* **This product is 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 human peripheral blood mononuclear cells (PBMCs) 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 cryovial with 70% ethanol, and wipe cryovial with lint-free lab wiper. Open cryovial under laminar flow hood and transfer cells into a 50 mL conical centrifuge tube.
4. Rinse the empty vial with 1 mL LYMPH-1, then slowly add drop wise to the 50 mL conical tube with thawed cells.
5. Slowly add LYMPH-1 drop wise to the 50 mL conical tube until the total volume of the cell suspension reaches 25 mL.
6. Centrifuge cell suspension at 400 x *g* for 10 minutes at room temperature.
7. Carefully remove the supernatant and save it in a second tube, leaving about 1 mL behind so as not to disturb the cell pellet.
8. Gently resuspend the cells in LYMPH-1, bringing the total volume up to 2 mL per vial of product. Count cells using a hemocytometer or automated cell counter.

   Note: If viability is lower than expected, re-spin at 100 x *g* for 10 minutes at room temperature and repeat steps 7 and 8 for an additional cell count.
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 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. 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 the peripheral blood mononuclear cells. The uses for this product 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 of 80% upon thawing from cryopreservation. Once shipping or storage issues have been eliminated, try re-spinning the cells at 100 x *g* and then re-counting. 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.

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 PBMCs 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 PBMCs.

I received fresh PBMCs and stored them in refrigerator upon receipt. How long are the cells viable?

Freshly isolated PBMCs will perform optimally when used within 0-3 hours upon receipt. They may be stored for 1-2 days after arrival at +2-8°C and still retain enough viability for any experimentation needs.

I have too many PBMCs in the vial to use at one time. Is it possible to use part of the vial contents and re-freeze the remainder of the PBMCs for future use?

We do not recommend re-freezing thawed peripheral blood mononuclear cells (PBMCs). The cells are for single thaw and use only. The PBMCs contain multiple cell sub-types that can grow, differentiate, attach, or remain in suspension. If you re-freeze PBMC vial contents, the ratio of different cell types will be different than what was reported on the certificate of analysis and the reported viability will be greatly reduced. Thawing and re-freezing Zen-Bio PBMCs would negate the warranty for any replacements, discounts, or refunds.

PATHOGEN TESTING

Samples from each donor are tested via PCR to confirm non-reactivity for HIV-1, HIV-2, syphilis, Chagas, West Nile virus, 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). Always wear gloves and work behind a protective screen when handling primary human cells.