Human Mast Cell Care Manual

INSTRUCTION MANUAL ZBM0132.00

SHIPPING CONDITIONS

Human Mast Cells

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

- Lymphocyte Medium
  +4°C Expires 30 days from ship date -20°C Expires 6 months from ship date
- Cryopreserved cells: Vials of cryopreserved cells are to be stored in vapor phase nitrogen (-150°C to -190°C) immediately upon arrival

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

ORDERING INFORMATION AND TECHNICAL SERVICES

ZenBio, Inc.
3920 South Alston Avenue
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-DMC-F	HUMAN ADULT MAST CELLS, 500,000 VIABLE 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 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 mast cell viability depends greatly on the use of suitable media, reagents, and sterile plastic wear. If these parameters are not carefully observed, cell growth may be slower than expected.

INTRODUCTION

ZenBio, Inc. cryopreserved primary human mast cells are isolated from the dermis of donated skin from consenting donors or human colon obtained via the gift of organ donation from donor tissue that is not suitable for organ transplantation. Each competent (living) volunteer adult skin donor has signed an Institutional Review Board (IRB) 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 protocols in compliance with ethical regulations. Each colon donor has confirmed documentation on file allowing for non-clinical research use of any non-transplantable organs or tissues in compliance with ethical regulations. All samples are collected and processed in the United States.

QUALITY CONTROL

Quality control tests are performed for each lot of Human Peripheral Blood Mononuclear cells. The mast cells are assessed for viability (>80%) and characterized by their cell surface markers (CD117, FcεR1a, Chymase, and Tryptase) via flow cytometry. Viability and cell surface marker data is expressed as percentage positive and presented on the certificate of analysis for each lot of cells. In addition, cells have been tested for common blood borne pathogens and microbial contamination including HIV-1, HIV-2, Hepatitis B, Hepatitis C, and syphilis.

MATERIALS PROVIDED FOR EACH CATALOG ITEM

- **Cryopreserved Human Mast Cells**
  - Cat # SER-DMC-F
  - Cryopreserved vial containing 500,000 viable human mast cells (store in vapor phase liquid nitrogen upon receipt- any other storage negates the warranty)

MEDIA COMPOSTIONS

Lymphocyte Medium catalogue # LYMPH-1 / LYMPH-1-50	**Expiration Dates**
• RPMI 1640 • L-Glutamine • Fetal Bovine Serum (US Origin) • DNAse I • Penicillin • Streptomycin • Amphotericin B	• If placed at +4°C upon arrival, the media is stable 30 days from the ship date *Use the +4°C expiration date listed on the label.* • If stored at -20°C upon arrival, it is stable 6 months after the ship date *Use the -20°C expiration date listed on the label.* • Upon thawing, add fresh antibiotics at 1% volume when you are ready to use. • The media will now expire 30 days after the thawing date

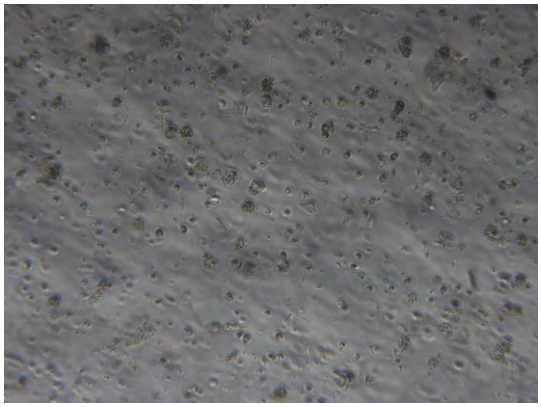
THAWING PROCEDURES: Cryopreserved Human Mast Cells _____

Please note: Human Primary Mast Cells are for single use only

Lymphocyte Medium is not a culture or growth medium.

1. Warm the Lymphocyte Medium (catalogue # LYMPH-1 OR LYMPH-1-50) to 37°C. Prepare all your sterile pipets and vessels.
2. Remove cells from liquid nitrogen and place **immediately** into a 37°C water bath with agitation. Be careful not to submerge the cap of the vial into water. For best results, the thawing step should not take more than 2 minutes. Stop thawing when there is still some ice in the vials. Rinse the outside of the vials with 70% ethanol before opening.
3. Aseptically transfer the cells to a sterile 50 mL tube.
4. Rinse the vial with 1 mL of Lymphocyte Medium. Then slowly add medium drop wise to the cells in the 50 mL conical tube while gently swirling the tube.
5. Add medium drop wise to the 50 mL tube until the total volume reaches 25 mL.
6. Centrifuge at 400x g at room temperature for 10 minutes.
7. Carefully remove the supernatant and save in a second tube, leaving 1mL behind as not to disturb the pellet.
8. Gently resuspend the cells up to a volume of 1 mL per vial of product thawed. Count the number of cells. If viability is lower than expected, re-spin at 100x g for 10 minutes and recount.
9. Gently resuspend cells to desired concentration as per your protocol.

HUMAN MAST CELLS Morphology:



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 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 Mast Cells?** No. Our Lymphocyte Medium is NOT a culture or a growth medium. It is a medium designed to successfully thaw mast cells with high viability. Human primary Mast cells are for single one-time use only.

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, Hepatitis B, Hepatitis C and syphilis. However, since we cannot test all pathogens, please treat the product as a potentially infectious agent at Biosafety Level 1 (BSL-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 human mast cells.

PATHOGEN TESTING

Each lot of primary cells is tested via PCR and found non-reactive to viral DNA from HIV and hepatitis B and viral RNA from Hepatitis C using US Food and Drug Administration (FDA) tests. However, 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 (BSL-1) or higher.

Always wear gloves and work behind a protective screen when handling primary human cells.

REFERENCES

- Siiskonen H, Scheffel J. Isolation and Culture of Human Skin Mast Cells. Methods Mol Biol. 2020;2154:33-43. doi: 10.1007/978-1-0716-0648-3_4. PMID: 32314206.