Human CD8+T Cell Care Manual

INSTRUCTION MANUAL ZBM0068.02

SHIPPING CONDITIONS

- **Human CD8+T Cells, cryopreserved**

Cryopreserved human CD8+T 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.**

STORAGE CONDITIONS

- **Cryopreserved cells:** Vials of frozen CD8+T cells are to be stored in vapor phase nitrogen (-150°C to -190°C).
- **Lymphocyte Medium**: Store 2-8°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.
3200 East NC-54 Suite 100
PO Box 13888
Research Triangle Park, NC 27709
U.S.A.

Telephone	(919) 547-0692
Facsimile (FAX)	(919) 547-0693
Toll free (continental US only)	1-866-ADIPOSE 1-(866)-234-7673
Electronic mail (e-mail)	information@zenbio.com
World Wide Web	http://www.zenbio.com

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.

INTRODUCTION

CD8+ T cells are recognized as T_C cells once they become activated and are generally classified as having a pre-defined cytotoxic role within the immune system. However, CD8+ T cells also have the ability to make some cytokines. Antigen presentation stimulates T cells to become either "cytotoxic" CD8+ cells or "helper" CD4+ cells. A cytotoxic T cell belongs to a sub-group of T lymphocytes that are capable of inducing the death of infected somatic or tumor cells; they kill cells that are infected with viruses (or other pathogens), or are otherwise damaged or dysfunctional. Most cytotoxic T cells express T-cell receptors) that can recognize a specific antigenic peptide bound to Class I MHC molecules, present on all nucleated cells, and a glycoprotein called CD8, which is attracted to non-variable portions of the Class I MHC molecule. The affinity between CD8 and the MHC molecule keeps the T_C cell and the target cell bound closely together during antigen-specific activation.

CD8+ Cytotoxic T cells are derived from fresh peripheral blood of healthy donors and produced at the Zen-Bio facility. Peripheral blood CD8+ Cytotoxic T cells are isolated from mononuclear cells using positive immunomagnetic selection for CD8+ cells. Immediately after isolation, the freshly prepared CD8+ Cytotoxic T cells are cryopreserved using a serum-based cryopreservation medium. Each vial contains 5 million cells per vial.

QUALITY CONTROL

Quality control tests are performed for each lot of CD8+T 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 some common blood borne pathogens and microbial contaminants.

MATERIALS PROVIDED FOR EACH CATALOG ITEM

- **Cryopreserved Naive CD8 T_C Cells from Normal Peripheral Blood**
  Catalog # SER-PBCD8+KT-N-F
  Frozen vial containing 5.0 million cells/vial

- **Cryopreserved CD8 T_C Cells from Normal Human Peripheral Blood**
  Catalog # SER-PBCD8+KT-F
  Frozen vial containing 5.0 million cells/vial

- **Cryopreserved Activated CD8 T_C Cells from Normal Peripheral Blood**
  Catalog # SER-PBCD8+KT-A-F
  Frozen vial containing 5.0 million cells/vial

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 $CD8^+$T cells

1. Warm the appropriate medium that will be used to thaw the cells 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. Gently resuspend the cells up to a volume of 2 mL (2 mL per vial of product). Count the number of cells. If count is lower than expected, centrifuge the wash that was saved at a higher speed, count and combine if necessary.
8. Gently resuspend cells to desired concentration.

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 cells?** 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. 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).

7. **My cells are not attaching or proliferating. What is wrong?** Nothing is wrong. We recommend that you thaw and use the cells directly. 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, 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.

株式会社ケー・エー・シー
試薬事業部
〒661-0978　兵庫県尼崎市久々知西町2丁目1-20

（お問合せ先）
TEL：06-6435-9747　FAX：06-6435-9748
URL：http://www.kacnet.co.jp/
E-mail：cs-info@kacnet.co.jpC