Human CD4+T Cell Care Manual

INSTRUCTION MANUAL ZBM0067.05

SHIPPING CONDITIONS

All US and Canada orders are shipped via Federal Express Priority service and are usually received the next day. International orders are shipped via FedEx or DHL service using dry ice or a dry vapor shipper if transit time will exceed 3 days. Primary human cells are very sensitive to extended times (> 3 days) transported using dry ice. Please inquire for dry vapor shipper availability if your transit time will exceed 3 days. Cells should always be stored in liquid nitrogen vapor phase immediately upon arrival.

STORAGE CONDITIONS

- **Cryopreserved cells:** Vials of frozen CD4+ cells are to be stored in vapor phase nitrogen (-150°C to -190°C) immediately upon arrival
- **Lymphocyte Medium**: Media: 30 days from ship date +4°C
  6 months from ship date -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.

ORDERING INFORMATION AND TECHNICAL SERVICES

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THIS MANUAL IS SUITABLE FOR USE WITH THE FOLLOWING PRODUCTS:

SER-CD4+T	Cryopreserved CD4+T Cells from Normal Human Peripheral Blood, 5 million viable cells/vial
SER-PBCD4+TH-N-F	Cryopreserved Naive CD4+ T_H Cells from Normal Peripheral Blood, 1 million viable cells/vial
SER-CBCD4+T	Cryopreserved CD4+T Cells from Umbilical Cord Blood, 5 million 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 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 stored and thawed according to Zen-Bio protocols without amendment or substitution.

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

T Cells have a key function in the adaptive immune system. They can either promote growth and differentiation of other immune cells or show suppressive function and down-regulate immune reactions.

CD4+ T lymphocytes play a central role in regulating the cell mediated immune response to infection. These cells are often known as "helper" T cells, as they act on other cells of the immune system to promote various aspects of the immune response, including immunoglobulin isotype switching and affinity maturation of the antibody response, macrophage activation, and enhanced activity of natural killer (NK) cells and cytotoxic T cells (CTL).

CD4+ T cells act by releasing cytokines in response to antigenic stimulation. Cytokines are soluble intercellular messenger molecules, which interact with specific receptor molecules on their "target" cells. The release of cytokines allows cells of different types to "talk" to each other in the on-going immune response. One of the major effector functions of CD4+ T cells is in the activation of macrophages. Macrophage activation plays an important role in enhancing bacterial killing at sites of infection. Applications for $CD4^+$T cells include: drug testing and drug discovery, assessing immune response, toxicology, genetic studies, inflammation, and autoimmune diseases.

The $CD4^+$ T Cells are produced using an indirect magnetic labeling system for the isolation of untouched $CD4^+$ T helper cells from human peripheral blood mononuclear cells (PBMCs) at the Zen-Bio facility. Non-$CD4^+$ T cells, *i.e.,* $CD8^+$ T cells, B cells, Natural Killer (NK) cells, dendritic cells, monocytes, granulocytes, and erythroid cells, are screened for CD8, CD14, CD15(16) CD19, CD56, and glycophorin A. CD4+ T lymphocyte cells are isolated from normal peripheral blood using negative immunomagnetic selection directed against the CD4 surface antigen. Negative selection is preferred with immune cell isolations to avoid activation of the cells by the antibody.

ZenBio, Inc. CD4+ cells are isolated from anticoagulated blood collected in the United States from a volunteer adult donor who has signed an Institutional Review Board (IRB) or US Food and Drug Administration (FDA) 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 Good Laboratory Practices (GLP) protocols in compliance with ethical regulations.

QUALITY CONTROL

Each lot of CD4+T cells is assessed for viability and characterized by CD4 cell surface marker via flow cytometry. Population distributions are 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 >80%. Each lot is also tested and found non-reactive to viral DNA from Hepatitis B and viral RNA from HIV 1, HIV-2 and Hepatitis C. Hepatitis B Surface antigen (HBsAg) and HIV antibody (Ab), ZIKA Virus and STS (Syphilis) are also found non-reactive by US Food and Drug Administration (FDA) licensed tests.

MATERIALS PROVIDED FOR EACH CATALOG ITEM

- **Cryopreserved CD4+T Cells from Normal Human Peripheral Blood**
  Catalog #: SER-CD4+T
  Frozen vial containing 5.0 million cells/vial

- **Cryopreserved Naive CD4+ T_H Cells from Normal Peripheral Blood**
  Catalog #: SER-PBCD4+TH-N-F
  Frozen vial containing 1.0 million cells/vial

- **Cryopreserved CD4+T Cells from Umbilical Cord Blood**
  Catalog #: SER-CBCD4+T
  Frozen vial containing 5.0 million cells/vial

LYMPHOCYTE MEDIUM COMPOSITION

Recommended product for thawing cells.

Cat# LYMPH-1 (100ml); LYMPH-1-50 (50ml)

Medium Composition	Expiration Dates
RPMI 1640 L-Glutamine Fetal Bovine Serum (FBS) DNAse I Penicillin Streptomycin Amphotericin B	• If placed at 4°C upon arrival, the media is stable until the expiration date on the bottle label which is 30 days from the shipping date. • If stored at -20°C upon arrival, the media is stable for 6 months. Add fresh antibiotics when you are ready to use. The media will expire 30 days after the thaw date.

THAWING CRYOPRESERVED CD4^{+}T cells

1. Warm the Lymphocyte Medium (cat# LYMPH-1) 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 sterile 50mL conical tube.
4. Rinse the vial with 1 mL of medium. Then slowly add the media 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. **Can I re-freeze any cells I do not need?** No. This product is for thawing and use one time only.

4. **Do you test for pathogens? Which ones?** Yes. Samples from each donor are tested via 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 culture as a potentially infectious agent at Biosafety Level 1 or higher.

5. **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.

6. **Do you have any protocols for ways to use the cells?** No. We do not provide any protocols for the use of the cells. The uses for this product are too varied to provide a comprehensive protocol suitable for each experiment.

7. **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).

8. **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

Each lot is tested via PCR and found non-reactive to viral DNA from Hepatitis B and viral RNA from HIV 1, HIV-2 and Hepatitis C. Hepatitis B Surface antigen (HBsAg) and HIV antibody (Ab), ZIKA Virus and STS (Syphilis) are also found non-reactive by US Food and Drug Administration (FDA) licensed tests. 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 or higher.. Always wear gloves and work behind a protective screen when handling primary human cells.