



Human CD3+T Care Manual

INSTRUCTION MANUAL ZBM0086.01

SHIPPING CONDITIONS

- Human CD3+T, cryopreserved

Cryopreserved human CD3+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 CD3+T cells are to be stored in vapor phase nitrogen (-150°C to -190°C).
- **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.

ORDERING INFORMATION AND TECHNICAL SERVICES

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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

CD3 is initially expressed in the cytoplasm of pro-thymocytes, the stem cells from which T-cells arise in the thymus. The pro-thymocytes differentiate into common thymocytes, and then into medullary thymocytes, and it is at this latter stage that CD3 antigen begins to migrate to the cell membrane. The antigen is found bound to the membranes of all mature T-cells, and in virtually no other cell type, although it does appear to be present in small amounts in Purkinje cells. This high specificity, combined with the presence of CD3 at all stages of T-cell development, makes it a useful immunohistochemical marker for T-cells in tissue sections. The antigen remains present in almost all T-cell lymphomas and leukaemias, and can therefore be used to distinguish them from superficially similar B-cell and myeloid neoplasms. Because CD3 is required for T-cell activation, drugs (often monoclonal antibodies) that target it are being investigated as immunosuppressant therapies (e.g., orelizumab) for type 1 diabetes and other autoimmune diseases.

CD3 is expressed on all T cells and CD56+ NKT cells, and is associated with the T cell receptor. 70–80% of human peripheral blood lymphocytes and 65–85% of thymocytes are CD3+

CD3+ T cells are isolated from normal peripheral blood using negative immunomagnetic selection directed against the CD3 surface antigen. Negative selection is preferred with immune cell isolations to avoid activation of the cells by the antibody.

QUALITY CONTROL

Quality control tests are performed for each lot of CD3+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 CD3+T Cells from Normal Human Peripheral Blood

Catalog # SER-CD3-F

Frozen vial containing 5 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 CD3⁺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.