



Human CD56 Natural Killer (NK) Cell Care Manual

INSTRUCTION MANUAL ZBM0076.04

SHIPPING CONDITIONS

- **Human CD56 NK, cryopreserved**
- Cells are shipped using dry ice or dry vapor shipper (if the transit time will exceed 3 days). Orders are delivered via Federal Express or DHL courier. All US and Canada orders are shipped via Federal Express Priority service and are usually received the within 1-2 business days. International orders are usually received in 2-4 days. Alternate couriers and dry vapor shipper options are available if needed. Please inquire.

STORAGE CONDITIONS

- **Cryopreserved cells:** Vials of frozen CD56+ Natural Killer cells are to be stored in vapor phase nitrogen (-150°C to -190°C) IMMEDIATELY upon arrival.
- **Lymphocyte Medium Expiration Dates** (from the ship date):
30 days stored at 4°C 6 months stored at -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-PBCD56+NK-F	CD56 NATURAL KILLER CELLS FROM NORMAL HUMAN PERIPHERAL BLOOD , CRYOPRESERVED
SER-PBCD56-UNT-F	NORMAL HUMAN PERIPHERAL BLOOD NATURAL KILLER CELLS, CD56+, UNTOUCHED, CRYOPRESERVED, 5 MILLION CELLS/VIAL. NEGATIVE SELECTION,



LIMITED PRODUCT WARRANTY

This warranty limits our liability for 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 and storage conditions are followed. Cryopreserved human blood derived 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

Natural Killer cells are large granular lymphocytes that are a type of cytotoxic lymphocyte critical to the innate immune system. They are differentiated from the common lymphoid progenitor and are known to differentiate and mature in bone marrow, lymph node, tonsils, spleen, and the thymus where they then enter into circulation. NK cells express the surface marker CD56 in humans and provide rapid responses to virally infected cells. They respond to tumor formation and have the ability to recognize stressed cells in the absence of antibodies and major histocompatibility complex, allowing for a much faster immune reaction. Applications include analysis of receptor expression, cytokine secretion, or cell-cell interaction with other cells of the immune system.

Human peripheral blood mononuclear cells (PBMC's) are isolated from anticoagulated blood or a Leukopak collected from consented adult donors collected in the United States. Each volunteer adult donor 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.

Positively Selected CD56+ NK cells are directly isolated from mononuclear cells using direct immunomagnetic CD56 MicroBeads.

Negatively selected CD56+ Natural Killer cells are isolated from the PBMC's indirect immunomagnetic NK cell labeling to deplete the non-CD56+ NK cells.

Zen-Bio offers CD56+ Natural Killer cells in a phenotypically undifferentiated state. Immediately after isolation, the freshly prepared CD56+ NK cells are cryopreserved using an animal-free cryopreservation medium. Each vial contains 5 million viable cells per vial.

QUALITY CONTROL

Quality control tests are performed for each lot of CD56 Natural Killer cells for viability and cell surface markers via flow cytometry. Population distributions of cell surface markers are expressed as percentage positive and are presented on the certificate of analysis for each lot of cells. Cells have a guaranteed purity of >95% and a viability >80%. 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), HIV antibody (Ab), and STS (Syphilis) are also found non-reactive by US Food and Drug Administration (FDA) licensed tests. 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.



MATERIALS PROVIDED FOR EACH CATALOG ITEM _____

- - **Cryopreserved CD56 Natural Killer cells from Normal Human Peripheral Blood**
 - Catalog # SER-PBCD56+NK-F
 - Cryopreserved vial containing 5 million viable CD56+ cells/vial
 - Long term storage: vapor phase liquid nitrogen. Any other storage negates the warranty.

 - Normal Human Cryopreserved Peripheral Blood Natural Killer Cells, CD56+, UNTOUCHED, Cryopreserved, 5 million cells/vial. Negative Selection
 - Catalog # SER-PBCD56-UNT-F
 - Long term storage: vapor phase liquid nitrogen. Any other storage negates the warranty.

LYMPHOCYTE MEDIUM COMPOSITION _____

Recommended product.

**Cat# LYMPH-1 (100ml);
LYMPH-1-50 (50ml)**
RPMI 1640
L-Glutamine
Fetal Bovine Serum (FBS)
DNase I
Penicillin
Streptomycin
Amphotericin B

EXPIRATION DATES:

- If placed at 4°C upon arrival, the media is stable until the expiration date on the bottle label.
- 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 when stored at +4°C.

THAWING CRYOPRESERVED CD56 NATURAL KILLER CELLS

1. Warm the Lymphocyte medium (cat# LYMPH-1) 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 LYMPH-1 medium. Then slowly add drop wise to the cells in the 50 mL conical tube while gently swirling the tube.
5. Slowly add LYMPH-1 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 in the medium of your choice for your experiment.

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, 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 (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 peripheral blood CD56+ cells.
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 the cryopreserved state. 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. CD56+ cells do not attach to cultureware and do not proliferate.

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), HIV antibody (Ab), 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.