

FOR RESEARCH USE ONLY

Product Description

LifeNet Health's primary human Kupffer cells are isolated from donated human tissue, resulting from the generous gift of an individual or their family. The cells are isolated using a refined cell isolation technique resulting in high-quality cells suitable for a wide range of research applications.

Indications for Use

LifeNet Health's primary human Kupffer cells are for research use only. The cells are not intended for human use, for any *in vitro* diagnostic procedures, or for therapeutic procedures. Transfer or resale of any LifeNet Health cells or products is prohibited without the written consent of LifeNet Health.

Warnings and Precautions

Observe universal precautions when handling human-derived tissues and cells as they are potentially biohazardous. Refer to the guidelines set forth in Occupational Safety and Health Standards for handling blood, tissues, body fluids, or other potentially infectious materials. Follow institutional guidelines for the collection and disposal of all solid and liquid waste that has been in contact with these products.

Donor Screening and Testing

The tissue was deemed suitable for research use by LifeNet Health. A physician medical director evaluated the following donor variables to determine donor suitability: infectious disease test results, current donor medical history, behavioral risk assessment interview, physical assessment, relevant medical records, including previous medical history, laboratory test results, and autopsy or coroner reports (if performed).

All donors are tested for relevant infectious diseases. Testing is performed by laboratories that are registered with the U.S. Food and Drug Administration (FDA) and certified under the Clinical Laboratory Improvement Amendments of 1988 (CLIA). Test methods that are FDA-licensed, approved, or cleared for donor screening are used as available. The following test criteria were met for the donor of these cells:

Infectious Disease Testing	
Test	Acceptance Criteria
HBcAb: Hepatitis B Total Core Antibody	Negative/Non-Reactive
HBsAg: Hepatitis B Surface Antigen	Negative/Non-Reactive
HBV NAT: Hepatitis B Virus Nucleic Acid Test*	Negative/Non-Reactive
HCV NAT: Hepatitis C Virus Nucleic Acid Test	Negative/Non-Reactive
HCVAb: Hepatitis C Antibody	Negative/Non-Reactive
HIV-1 NAT: Human Immunodeficiency Virus Type 1 Nucleic Acid Test	Negative/Non-Reactive
HIV 1/2 Ab: Human Immunodeficiency Virus Types 1/2 Antibody	Negative/Non-Reactive
RPR/STS or Equivalent: Syphilis	Confirmatory Negative/Non-Reactive

*Not performed on donors recovered prior to December 16, 2016.

Storage Requirements

The distributor, intermediary and/or end-user is responsible for storing these cells under appropriate conditions prior to further distribution or use. LifeNet Health ships frozen cells in the vapor phase of liquid nitrogen (-135°C to -190°C). On receipt, immediately transfer frozen cells to storage in the vapor phase of liquid nitrogen (-135°C to -190°C) until ready for experimental use.

Final Product Testing

Each LifeNet Health primary human Kupffer cell lot is fully characterized to determine post-thaw results including cell viability and yield, and morphological integrity as well as cytokine induction by lipopolysaccharide (LPS) stimulation. Each cell culture is tested and determined negative for bacteria, yeast, and fungi. A Certificate of Analysis (CoA) is available for each lot and includes comprehensive donor history, histological images with pathology results, characterization data, and respective cell culture images.

Complaints and Returns

For further information or returns or to report a complaint, please contact your authorized distributor or LifeNet Health Client Services (available 24 hours a day) at 1-888-847-7831 (inside the U.S.) or 00+1-757-464-4761 ext. 2000 (outside the U.S.) and have the product code and lot number available (see CoA).

Human Kupffer Cell Protocols

It is important to read and understand the following instructions prior to use. Improper handling may adversely affect cell quality and performance.

Recommended supplies and reagents

Kupffer Cell (KC) Culture Media: Corning RPMI 1640 (cat. # 10-040-CV) + 10% FBS (such as Gemini Biologicals cat. # 100-106) + 1% Penicillin/Streptomycin solution (such as Gibco cat. # 15140-122)

Culture Vessels: Tissue culture treated plastic ware or collagen type 1 coated plastic ware

Thawing Procedure

Note: (Keep cells on ice and cold until CENTRIFUGE PROCEDURE)

1. In Biological Safety Cabinet (BSC): Place 9 mL of cold KC Culture Media in a 15 mL conical tube and keep on ice. Keep the rest of KC Culture Media at room temperature for steps in CENTRIFUGE PROCEDURE and PLATING PROCEDURE.
2. Hold cryovial(s) containing Kupffer cells in a 37°C water bath to thaw without submerging the cap in water (hold until only a sliver of ice remains, approximately 1 ½ -2 minutes).
3. Remove from water bath and clean exterior of vial(s) with 70% ethanol before placing into BSC.
4. In BSC: Transfer entire contents of the cryovial(s) into the 15 mL conical tube of cold KC Culture Media. Scale volume up for additional vials (ex. 5 vials into 50 mL). 15 mL conical tubes are recommended for individual vial thaw to achieve better post-thaw yield and viability.
5. In BSC: Remove 1 mL of the cell suspension from the 15 mL tube and use it to rinse the cryovial(s) to capture residual cells; return the 1 mL rinse to the 15 mL tube and recap tube.
6. In BSC: Gently invert the 15 mL conical tube 5-6 times to mix well.

**** Please note that human Kupffer cells do not proliferate in culture and cannot be passaged; therefore, the appropriate number of vials must be thawed to obtain the desired number of cells.****

Centrifuge Procedure

1. Centrifuge cells at 500 x g for 5 minutes.
2. In BSC: After centrifugation, gently aspirate supernatant then re-suspend pellet immediately in appropriate volume of fresh cold KC Culture Media for cell counting.

Plating Procedure

1. Determine cell number and viability using lab standard methods and procedures.
2. In BSC: Add additional KC culture Media to bring the cells to a concentration of 0.2 – 0.4 X10⁶ cells/mL or other desired concentration.
3. In BSC: Dispense the desired cell number into the culture vessel and swirl gently to distribute.
4. Place culture vessels in humidified 37°C incubator @ 5% CO₂.
5. In BSC: After 4-6 hours, carefully aspirate the media and replace with an equal volume of fresh warm KC Culture Media.

Cell Culture Maintenance Procedure:

1. Adult human Kupffer cells can be maintained up to 7 days.
2. In BSC: Aspirate and replace KC Culture Media every day or as required by the experiment.
3. Continue this schedule until the conclusion of the experiment.