FOR NON-CLINICAL 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 humanderived 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

Donor authorization for non-clinical research use of these cells was appropriately obtained and documented by LifeNet Health. All donors are tested and confirmed negative or non-reactive for the following infectious diseases: Human Immunodeficiency Virus (HIV), Hepatitis B Virus (HBV), Hepatitis C Virus (HCV), Syphilis, and Toxoplasmosis.

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 on dry ice or in the vapor phase of liquid nitrogen (-135°C to -190°C) depending on the quantity of vials being shipped. 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 postthaw 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)

- 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 $1\frac{1}{2}$ -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.
- 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 $0.2 0.4 \times 10^6$ 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.

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