

## Human Kupffer Cells (HKC)

### Instructions for Use

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#### I. Required Materials

**NOTE:** Use universal precautions and aseptic technique at all times when handling human cells

1. HKC (Lonza catalog number HLKC)
2. RPMI 1640 Medium (HKC culture medium) (Lonza catalog number 12-702Q or equivalent)

3. Lonza 10K/10K stock Penicillin/Streptomycin solution (Lonza catalog number 17-602E or equivalent)
4. Biological Safety Cabinet (BSC)
5. Tissue culture treated plasticware or collagen type 1 coated plasticware
6. 37°C water bath
7. Fetal bovine serum (Gemini Biologicals catalog number. #100-106 or equivalent)

#### II. Unpacking and Storage Instructions

1. Check all containers for leakage or breakage.
2. For cryopreserved cells, remove cryovials from the dry ice packaging and immediately place into liquid nitrogen storage. Alternatively, thaw and use the cells immediately. If no dry ice remains, please contact Customer Service.

**NOTE:** Using media or reagents other than what is recommended will void the cell warranty. Please contact Scientific Support if you need help selecting media and/or reagents.

#### III. Preparation of Medium

1. Add 10% FBS and 1% penicillin/streptomycin to the RPMI 1640 basal medium.

#### IV. Thawing of Cells / Initiation of Culture Process

**NOTE:** Keep cells on ice and cold until seeding on culture plates. Kupffer cells easily attach to the walls of the conical tube at 37°C. Therefore, use of pre-warmed medium is not recommended at this step.

1. IN BSC: Place 9mL of 4°C HKC culture medium in a 15mL conical tube and keep on ice.
2. Hold HKC cryovial(s) 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 15mL conical tube of cold HKC culture medium. Scale volume up for additional vials (ex. 5 vials into 50 mL).
5. IN BSC: Remove 1.0 mL of the cell suspension from the 15mL tube and use it to rinse the cryovial(s) to capture residual cells; return the 1.0mL rinse to the 15mL tube and recap tube.
6. IN BSC: Gently invert the 15mL conical tube 5-6 times to mix well.

## V. Centrifuge Procedure

1. Centrifuge cells at 500 x g for 5 minutes at 4°C
2. IN BSC: After centrifugation, gently aspirate supernatant then re-suspend pellet in 2mL of fresh 4°C HKC culture medium.

## VI. Plating Procedure

Note: Kupffer cells are very sticky upon thaw, but become less sticky over time. Plating to collagen coated plates is not required, but may promote better attachment.

1. Determine cell number and viability using lab standard methods and procedures.
2. IN BSC: Add additional cold HKC culture medium to bring the cells to a concentration of 0.2 – 0.4 X10<sup>6</sup> 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 at 5% CO<sub>2</sub>.
5. IN BSC: After 4 - 6 hours, carefully aspirate the medium and replace with an equal volume of fresh HKC culture medium.

## VII. Maintenance

**NOTE:** Adult Human Kupffer Cells can be maintained up to 7 days.

1. IN BSC: Aspirate and replace HKC culture medium every day or as required by the experiment.

**NOTE:** Kupffer cells attach loosely and may be lost by vacuum aspiration. Use extreme caution during medium removal.

2. Continue this schedule until the conclusion of the experiment.

## VIII. Subculturing

**NOTE:** Human Kupffer Cells do not proliferate in culture and cannot be passaged. The appropriate number of vials must be thawed to obtain the desired number of cells.

## IX. Ordering Information

### Cryopreserved Cells

Cat. No.	Product	Size
HLKC	Human Cryopreserved Kupffer Cells	>500,000 viable cells/vial

### HKC Culture Medium (must be purchased separately)

Cat. No.	Product	Size
# 12-702Q or equivalent	Lonza RPMI 1640	500 mL
# 17-602E or equivalent	Lonza 10K/10K stock Penicillin/Streptomycin solution	100 mL

## X. Product Warranty

Cultures have a finite lifespan *in vitro*.

Lonza guarantees the performance of cells only if appropriate media and reagents are used exclusively and the recommended storage and use protocols are followed. Any modifications made to the recommended cell systems including the use of alternative media, reagents or protocols, will void cell and media performance guarantees. If you need assistance in selecting the appropriate media, reagents, or protocol, please contact Lonza Scientific Support.

## XI. Quality Control

For detailed information concerning QC testing, please refer to the Certificate of Analysis.

When placing an order or when contacting Scientific Support, please refer to the product numbers and descriptions listed above. For a complete listing of all Cell Biology Products, refer to the Lonza website or our current catalog. To obtain a catalog, additional information or Scientific

Support, you may contact Lonza by web, e-mail or telephone. Contact details are listed at the top of this document.

## **XII. Safety Statements**

**THESE PRODUCTS ARE FOR RESEARCH USE ONLY.** Not approved for human or veterinary use, for application to humans or animals, or for use in clinical or *in vitro* diagnostic procedures.

**WARNING: PRIMARY CELL PRODUCTS CONTAIN HUMAN SOURCE MATERIAL, TREAT AS POTENTIALLY**

**INFECTIOUS.** All human-sourced products should be handled at the biological safety level 2 to minimize exposure of potentially infectious products, as recommended in the CDC-NIH manual, [Biosafety in Microbiological and Biomedical Laboratories](#), 5<sup>th</sup> ed. If you require further information, please contact your site safety officer or Scientific Support.

HKC cells are manufactured by Samsara Sciences, Inc. San Diego, CA

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