

Human Cryopreserved Kupffer Cells 2e5 per vial (HLKC-200K), Human Cryopreserved Kupffer Cells 5e5 per vial (HLKC-500K), and Human Cryopreserved Kupffer Cells (HLKC)

Instructions for use

Table of contents:

Section	Description	Page
I	Required materials	1
II	Unpacking and storage instructions	1
III	Preparation of medium	1
IV	Thawing of cells / initiation of cell culture process	2
V	Centrifuge procedure	2
VI	Plating procedure	2
VII	Maintenance	2
VIII	Subculturing	2
IX	Ordering information	3
X	Product warranty	3
XI	Quality control	3
XII	Safety statements	3

For answers to frequently asked questions or references citing the use of these products, please visit Lonza's Knowledge Center:

<https://knowledge.lonza.com/>

I. Required materials

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

1. Human Cryopreserved Kupffer Cells 200K per vial (Lonza catalog number HLKC-200K), Human Cryopreserved Kupffer Cells 500K per vial (Lonza catalog number HLKC-500K), and

Human Cryopreserved Kupffer Cells (Lonza catalog number HLKC)

2. KuCM™ Kupffer Cell Culture BulletKit™ (MKC-500BK) Biological Safety Cabinet (BSC)
3. Tissue culture treated plasticware or collagen type 1 coated plasticware
4. 37°C water bath

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 Scientific Support at the email address provided in the header to this page.
3. Lonza's BulletKit™ Medium instructions: upon arrival, store basal medium at 2° - 8°C and SingleQuots™ Kit at -20°C in a freezer that is not self-defrosting. If thawed upon arrival, growth factors can be stored at 2° - 8°C and added to the basal medium within 72 hours of receipt. After the SingleQuots™ Kit Supplements are added to basal medium use within one month. Do not re-freeze.

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

III. Preparation of BulletKit™ Medium

1. Decontaminate the external surfaces of all supplement vials and the medium bottle with ethanol or isopropanol.
2. Aseptically open each supplement vial and add the entire contents to the basal medium with a pipette.
3. Rinse each cryovial with the medium. It may not be possible to recover the entire volume listed for each cryovial. Small losses, even up to 10%, should not affect the cell growth characteristics of the supplemented medium.
4. Transfer the label provided with each kit to the basal medium bottle being supplemented. Use it to record the date and amount of each supplement added. We recommend that you place the completed label over the basal medium label (avoid covering the basal medium lot # and expiration date) to avoid confusion or possible double supplementation. After adding supplements, the complete medium has a shelf life of one month stored at 2 - 8°C in the dark.
Do not freeze medium.
5. Record the new expiration date on the label based on the shelf life.

NOTE: If there is concern that the sterility was compromised during the supplementation process, newly prepared medium may be re-filtered with a 0.2-micron filter to assure sterility. Routine filtration/re-filtration is not recommended.

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. Place 9 mL of 4°C completed KuCM™ Kupffer Cell Culture BulletKit™ in a 15 mL conical tube and keep on ice.
2. Hold 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. Transfer entire contents of the cryovial(s) while sliver of ice remains into the 15 mL conical tube of cold HKC culture medium. Scale volume up for additional vials (ex. 5 vials into 50 mL).

5. 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 15 mL tube and recap tube.
6. Gently invert the 15 mL conical tube 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 by angling the conical and aspirating along the side of the tube, taking care to not disturb the pellet. Re-suspend pellet in 0.2-0.5mL of fresh 4°C KuCM™ Kupffer Cell Culture BulletKit™.

VI. Plating procedure

NOTE: Kupffer cells are very sticky upon thaw, but become less sticky over time. Plating on 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 KuCM™ Kupffer Cell Culture BulletKit™ 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 at 5% CO₂.

VII. Maintenance

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

1. Change the medium with freshly warmed, complete KuCM™ Kupffer Cell Culture BulletKit™ every 1 – 2 days 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-200K	Human Cryopreserved Kupffer cells 2 x 10 ⁵ per vial	≥200,000 viable cells/vial
HLKC-500K	Human Cryopreserved Kupffer Cells 5 x 10 ⁵ per vial	≥500,000 viable cells/vial
HLKC	Human Cryopreserved Kupffer Cells	≥500,000 viable cells/vial

HLKC BulletKit™ Culture Medium

(must be purchased separately)

Cat. no.	Product	Size
MKC-500BK	KuCM™ Kupffer Cell Culture BulletKit™	Contains 500mL bottle and SingleQuots
MKC-500	KuBM™ Kupffer Cell Culture Basal Medium	500 mL bottle
MKC-500SQ	KuBM SingleQuots™ Kupffer Cell Culture Supplements and Growth Factors	SingleQuots

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*, 5thed. If you require further information, please contact your site safety officer or Scientific Support.

All trademarks belong to Lonza and are registered in USA, EU or CH, or belong to their respective third-party owners and are being used for informational purposes only. All third-party copyrights have been produced with permission or belong to Lonza.

©2021 Lonza.