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Cryopreserved Human & Rat Kupffer Cells

Description

Kupffer cells, also known as Browicz-Kupffer cells and stellate macrophages, are specialized macrophages that line the walls of the sinusoids in the liver, which form part of the reticuloendothelial system (RES) (also called mononuclear phagocyte system). They play a role in recycling dead blood cells and helping the liver respond to toxic substances in the blood stream.

Product	Catalog no.	Amount	Storage
Human Kupffer Cells	HUKCCS	1×10^6 viable cells/vial	Store in liquid nitrogen (vapor phase)
Rat Kupffer Cells	RTKCCS		

Product Use

For Research Use Only. Not for use in diagnostic procedures.

Important Information

Kupffer cell monocultures grow best in medium supplemented with at least 2% FBS (10% recommended) and **NO corticosteroids** (e.g. Dexamethasone, Hydrocortisone).

Safety Information

Read the Safety Data Sheets (SDSs) and follow the handling instructions. Wear appropriate protective eyewear, clothing, and gloves.

Culture Conditions

Media: Kupffer Maintenance Medium and Co-culture Maintenance Medium for co-culture with hepatocytes, or Kupffer Monoculture Medium for monoculture.

Culture Type: Adherent monoculture or adherent co-culture with hepatocytes

Recommended Substrate: Collagen type I

Temperature Range: 36°C to 38°C

Incubator Atmosphere: Humidified atmosphere of 5% CO_2

Prepare Media

Prepare Kupffer Thawing/Plating Medium

Kupffer Thawing/Plating Medium consists of Advanced DMEM supplemented with FBS and a cocktail solution of fetal bovine serum (FBS), penicillin-streptomycin, human recombinant insulin, GlutaMAX™-I supplement, and HEPES.

For 500 mL of Kupffer Thawing/Plating Medium, asceptically mix the following components:

Advanced DMEM	457 mL
FBS	25 mL
Thawing/Plating Cocktail – A	18 mL

Note: FBS and Thawing/Plating Cocktail – A are available as part of the Primary Hepatocyte Thawing and Plating Supplements (Cat. no. CM3000). Do **not** add Dexamethasone to the Kupffer Thawing/Plating Medium.

Prepare Kupffer Maintenance Medium

Kupffer Maintenance Medium consists of Advanced DMEM supplemented with FBS and a cocktail solution of penicillin-streptomycin, ITS+ (insulin, transferrin, selenium complex, BSA, and linoleic acid), GlutaMAX™-I supplement, and HEPES.

For 500 mL of Kupffer Maintenance Medium, asceptically mix the following components:

Advanced DMEM	455 mL
FBS	25 mL
Maintenance Cocktail – B	20 mL

Note: Maintenance Cocktail – B is available as part of the Primary Hepatocyte Maintenance Supplements (Cat. no. CM4000). Do **not** add Dexamethasone to the Kupffer Maintenance Medium.

Prepare Co-culture Maintenance Medium

Hepatocyte-Kupffer Co-culture Maintenance Medium consists of Kupffer Maintenance Medium without FBS.

For 500 mL of Kupffer-hepatocyte Co-culture Maintenance Medium, asceptically mix the following components:

Advanced DMEM	480 mL
Maintenance Cocktail – B	20 mL

Prepare Kupffer Monoculture Medium

Kupffer Monoculture Medium consists of RPMI 1640 Medium with GlutaMAX™-I supplement and HEPES (Cat. no. 72400), and supplemented with 10% FBS and 1X penicillin-streptomycin.

For 500 mL of Kupffer Monoculture Medium, asceptically mix the following components:

RPMI 1640 Medium	445 mL
FBS	50 mL
Penicillin-Streptomycin (100X)	5 mL

Co-culture of Kupffer Cells with Hepatocytes

The protocols below assume using 24-well plates for all seeding densities. Adjust seeding densities for other well formats appropriately.

Recover Kupffer Cells for Co-culture

1. Remove a vial of frozen Kupffer cells from liquid nitrogen storage and rapidly thaw in a 37°C water bath until a small amount of ice remains in the cryovial.
2. Transfer the contents of cryovial into a 15-mL conical tube containing 9 mL of cold (4°C) Kupffer Thawing/Plating Medium and place on ice. Alternatively, you may thaw up to 5 cryovials of Kupffer cells in a 50-mL conical tube containing 45 mL of cold medium.
   Note: Kupffer cells are very "sticky" at physiological temperature of 37°C. If the medium is warmed to 37°C, the Kupffer cells will attach to any substrate including the walls of the conical tube. Therefore, use of pre-warmed media is **not** recommended at this step.
3. Centrifuge the cells at 500 × *g* for 5 minutes.
4. Resuspend the pelleted cells (note that the pellet will be very small) in 1–2 mL of Kupffer Thawing/Plating Medium using a P1000 micropipettor. Serological pipette can also be used however this may lead to clumping of the cells.
5. Count the cells using the trypan blue exclusion assay.
6. Dilute the cells in Kupffer Thawing/Plating Medium to 0.2×10^6 to 0.4×10^6 cells/mL (for inflammatory Kupffer/Hepatocyte co-cultures) or to the desired density per internal protocol.
7. Plate 0.5 mL of cell suspension per well of a 24-well plate coated with collagen type I substrate.
8. Place the cells in a humidified 37°C/5% CO_2 incubator and allow them to attach for 4–6 hours.
9. After 4–6 hours of attachment, replace the medium with fresh Kupffer Thawing/Plating Medium.

10. After 24 hours, replace the medium with Kupffer Maintenance Medium and proceed with your experiement or co-culture with Hepatocytes.
    Note: To maintain Kupffer cell cultures, replace spent medium with Kupffer Maintenance Medium every 24–48 hours.

Prepare Hepatocytes for Co-culture

Fresh Hepatocytes: Prior to plating, subject fresh hepatocytes to a Percoll® purification step by using 50:50 v/v of 90% Isotonic Percoll®:Kupffer Thawing/Plating Medium. Centrifuge the cells at 100 × *g* for 10 minutes. If the cell recovery is too low, you may increase the *g* force to 120 × *g*. Expect to see an increase in viability and a decrease in yield. This step is required to remove residual Kupffer cells.

Cryopreserved Hepatocytes: For cryopreserved hepatocytes, you may omit the Percoll® step if high quality platable human or rat hepatocytes (e.g., Gibco® Transporter or Induction Qualified Hepatocytes) are used. To enhance viability prior to plating, we recommend thawing hepatocytes in Hepatocyte Thaw Medium (Cat. no. CM7500).

90% Isotonic Percoll® Medium: Dilute Percoll® Medium (GE Healthcare Lifesciences, Cat. no. 17-0891-01) with 10% Dulbecco's Phosphate Buffer Solution (10X) (Cat. no. 14200-075).

Recommended Rat and Human Hepatocyte Seeding Density:
Freshly isolated hepatocytes: 24-well plate, 0.6×10^6 cells/mL
Cryopreserved hepatocytes: 24-well plate, 0.8×10^6 cells/mL

Co-culture with Hepatocytes

Plate Kupffer cells 24 hours prior to plating hepatocytes for best results. However, you may also plate hepatocytes 4–48 hours after Kupffer cells are plated.

1. Seed hepatocytes on top of Kupffer cells following standard protocol for plating hepatocytes using Kupffer Thawing/Plating Medium.
2. After allowing hepatocytes to attach for 4–6 hours, replace the medium with Co-culture Maintenance Medium.
3. Allow the hepatocytes to culture for 24 hours prior to conducting experiments. Do **not** apply a matrix overlay (e.g., Geltrex® matrix) in co-cultures.

Kupffer Cell Monoculture

Recover Kupffer Cells for Monoculture

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2. Transfer the contents of cryovial into a 15-mL conical tube containing 9 mL of cold (4°C) Kupffer Monoculture Medium and place on ice. Alternatively, you may thaw up to 5 cryovials of Kupffer cells in a 50-mL conical tube containing 45 mL of cold medium.
   Note: Kupffer cells are very "sticky" at physiological temperature of 37°C. If the medium is warmed to 37°C, the Kupffer cells will attach to any substrate including the walls of the conical tube. Therefore, use of pre-warmed media is **not** recommended at this step.
3. Centrifuge the cells at 500 × *g* for 5 minutes.
4. Resuspend the pelleted cells (note that the pellet will be very small) in 1–2 mL of Kupffer Monoculture Medium using a P1000 micropipettor. Serological pipette can also be used however this may lead to clumping of the cells.
5. Count the cells using the trypan blue exclusion assay.
6. Dilute the cells in Kupffer Monoculture Medium to 0.2×10^6 to 0.4×10^6 cells/mL or to the desired density per internal protocol.
7. Plate 0.5 mL of cell suspension per well of a 24-well plate coated with collagen type I substrate.
8. Place the cells in a humidified 37°C/5% CO_2 incubator and allow them to attach for 4–6 hours.
9. After 4–6 hours of attachment, replace the medium with fresh Kupffer Monoculture Medium.
10. After 24 hours, replace the medium with Kupffer Monoculture Medium and proceed with your experiement.
    Note: To maintain Kupffer cell cultures for longer (1–2 weeks), replace spent medium with Kupffer Maintenance Medium every 24–48 hours.

Kupffer Cell Activation

Add lipopolysaccharide (LPS, 1 µg/mL) to culture medium to activate Kupffer cells for 2–24 hours (depending on experimental design) prior to experiment in either Kupffer cell monoculture or co-culture to mimic liver inflammation.

LPS activation changes happen fairly quickly, and morphological changes in the cells can be observed in less than 2 hours.

1–4 hours: Cells start out darker and more square-like (rat) or elongated (human), then become spindle-like with longer, thin cytoplasmic projections and may appear dendritic-like. Cells exhibit high level of motility by migrating around the plate.

4–8 hours: Cells return to a rounder morphology.

8–24 hours: Cells flatten and their cytoplasm enlarges, creating large round cells with many vacuoles inside (macrophage-like). Cells exhibit lower motility.

Related Products

Product	Cat. no.
Advanced DMEM	12491
Primary Hepatocyte Thawing and Plating Supplements	CM3000
Primary Hepatocyte Maintenance Supplements	CM4000
Fetal Bovine Serum, Certified, US Origin	16000
RPMI 1640 Medium, GlutaMAX™, HEPES	72400
Penicillin-Streptomycin (5000 U/mL)	15070
DPBS (10X), no Calcium, no Magnesium	14200
Collagen I, Coated Plate 24 Well	A1142802

Explanation of Symbols and Warnings

The symbols present on the product label are explained below:

				
Temperature limitation	Use by	Batch code	Catalog number	Manufacturer

Limited Product Warranty

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