User Guide:

Thawing and Plating Cryopreserved Kupffer Cells

Product Information

Kupffer Cells are cryopreserved immediately after isolation (P0), preserving their viability and phenotype. Cell purity is assessed by Immunofluorescence analysis of CD68 expression. These ready-to-use cryopreserved cells are ideal for a range of applications, including disease modeling, metabolism research, and drug-induced liver injury (DILI) studies. In culture, the cells attach to both collagen-coated plates in monoculture and to pre-plated hepatocytes in co-culture systems. For optimal performance, it is recommended to use the cells with their dedicated medium kits: Kupffer Cell Thawing Medium Kit (LKCT-500K), Kupffer Cell Plating Medium Kit (LKCP-500K), Kupffer Cell Maintenance Medium Kit (LKCM-500K).

Cat. No.	Product Description	Size (Cells)
CSD-LKC-05	Cryopreserved SD Rat Kupffer Cells	0.5 million
CCD-LKC-05	Cryopreserved CD-1 Mouse Kupffer Cells	0.5 million
CCB-LKC-05	Cryopreserved C57BL/6N Mouse Kupffer Cells	0.5 million
CBD-LKC-05	Cryopreserved Beagle Dog Kupffer Cells	0.5 million
CCY-LKC-05	Cryopreserved Cynomolgus Monkey Kupffer Cells	0.5 million
CBM-LKC-05	Cryopreserved Bama Minipig Kupffer Cells	0.5 million

Storage & Shelf Life

Stable for 5 years at ≤ –150°C.

Thawing Protocol

1. The complete Kupffer Cell Thawing Medium and Plating Medium should be used at 4 °C without pre-warming to maximize cell recovery. The complete Maintenance Medium should be pre-warmed to 37 °C prior to culturing Kupffer Cells. For detailed instructions, please refer to ***Kupffer Cell Culture Medium Kit Datasheet***.
2. Transfer 15 mL of cold complete Kupffer Cell Thawing Medium to a sterile 50 mL centrifuge tube.
3. Take cryovial out of the liquid nitrogen (transport on dry ice or in liquid nitrogen).
4. Thaw cells for approx. 2minutes at 37 °C in the water bath. Remove the vial from the water bath while a small amount of ice remains to prevent over-thawing..
5. Shake gently. When the cells pull away from the vial wall, transfer the content of vial into the cold complete Kupffer Cell Thawing Medium.
6. Add 1 mL of complete Kupffer Cell Thawing Medium to the vial to wash any remaining cells from the vial(s).
7. Spin down at 300 x g for 5 minutes at 4°C to pellet the Kupffer cells.
8. Carefully remove the supernatant without disturbing the pellet. Resuspend the cell pellet in 1 mL of complete Kupffer Cell Plating Medium.
9. Determine the total cell count and the number of viable cells using the Trypan Blue exclusion method.
10. Dilute the cells to the desired number of viable cells/mL (appropriate cell number is assay dependent- recommended 4-6x10^4 cells/cm^2) with complete Kupffer Cell Plating Medium.
11. Add an appropriate volume of diluted cells to collagen-coated cell culture plates as follows:

    6-Well plate: 2 mL/well (requires a total volume of 12 mL per 6-Well plate)

    12-Well plate: 1 mL/well (requires a total volume of 12 mL per 12-Well plate)

    24-Well plate: 0.5 mL/well (requires a total volume of 12 mL per 24-Well plate)

    48-Well plate: 0.2 mL/well (requires a total volume of 10 mL per 48-Well plate)

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96-Well plate: 0.1 mL/well (requires a total volume of 10 mL per 96-Well plate)

12. Gently shake the plates in a back-and-forth and side-to-side manner to evenly distribute the cells. Avoid any circular movement, as this will cause the cells to unevenly pool in the center of the plates.
13. Carefully place the plates into a 37°C, 5% CO_2, saturating humidity incubator to allow the cells to attach.
14. After 24h of cell adhesion, replace the pre-warmed complete Kupffer Cell Maintenance Medium and proceed with the experiment. If necessary, replace the Maintenance medium every 2-3 days.

Related Products

Cat. No.	Product Description	Size	Store at
LKCT-500K	Kupffer Cell Thawing Medium Kit	500 mL/Kit	
LKCT-500b	Kupffer Cell Thawing Basal Medium (KCTM-b)	500 mL	4℃
LKCT-500s	Kupffer Cell Thawing Supplement (KCTM-s)	for 500 mL	-20℃
LKCP-500K	Kupffer Cell Plating Medium Kit	500 mL/Kit	
LKCP-500b	Kupffer Cell Plating Basal Medium (KCPM-b)	500 mL	4℃
LKCP-500s-Ⅰ	Kupffer Cell Plating Supplement Ⅰ (KCPM-s Ⅰ)	for 500 mL	-20℃
LKCP-500s-Ⅱ	Kupffer Cell Plating Supplement Ⅱ (KCPM-s Ⅱ)	for 500 mL	-20℃
LKCM-500K	Kupffer Cell Maintenance Medium Kit	500 mL/Kit	
LKCM-500b	Kupffer Cell Maintenance Basal Medium (KCMM-b)	500 mL	4℃
LKCM-500s	Kupffer Cell Maintenance Supplement (KCMM-s)	for 500 mL	-20℃
Col-Coated-6w	Collagen I Coated 6-Well Plate	1 Plate	4℃
Col-Coated-12w	Collagen I Coated 12-Well Plate	1 Plate	4℃
Col-Coated-48w	Collagen I Coated 48-Well Plate	1 Plate	4℃
Col-Coated-96w	Collagen I Coated 96-Well Plate	1 Plate	4℃
Col-Coated-384w	Collagen I Coated 384-Well Plate	1 Plate	4℃

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