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Thawing & Plating Cryopreserved Human Hepatic Kupffer Cells

This protocol is suitable for the handling of cryopreserved Kupffer cells. Please read through this entire protocol before attempting this procedure. The health of the cells is dependent upon following the protocol carefully.

Procedure for Thawing and Plating Cryopreserved Kupffer Cells

Note: Handle gently and quickly to maintain viability. Collagen I coated culture ware is required. DO NOT pre-warm medium to thaw cells.

1. Place vial in a 37°C water bath, hold and rotate vial gently until the contents are completely thawed. Remove the vial from the water bath immediately, wipe dry, rinse the vial with 70% ethanol and transfer to a biological safety cabinet. Remove cap, being careful not to touch the interior threads with fingers.
1. Using a pipette, gently transfer contents of vial to a 15 mL conical tube containing 9 mL of cold (4°C) Kupffer Cell Plating Medium (MCKP250) and place the tube on ice.

   Note: Kupffer cells easily attach to the walls of the conical tube at 37°C. Therefore, use of pre-warmed media is not recommended at this step.
2. Centrifuge tube at 500xg for 5 minutes. After centrifugation, aspirate medium and re-suspend cell pellet in 1mL cold Kupffer Cell Plating Medium (MCKP250).

   Note: the pellet will be very small. Resuspend using a P1000 pipettor, as resuspension using a serological pipette may lead to clumping of the cells.
3. Count the cells using the Trypan Blue Exclusion Assay.
4. Dilute the cells in warm Kupffer Cell Plating Medium (MCKP250) to 400,000 cells/mL.
5. Plate 100,000 cells/cm^2 on culture ware coated with collagen type I, see table 1.

Table 1.

Well Format	cm^2 per well	# Cells Per Well	Total # Cells Per Plate
96	0.32	32,000	3.07×10^6
48	1.02	102,000	4.89×10^6
24	1.94	194,000	4.66×10^6
12	3.87	387,000	4.64×10^6
6	9.62	962,000	5.77×10^6

6. Place the cells in a humidified 37°C/5% CO_2 incubator and allow them to attach for 4–6 hours.

7. After attachment, replace the medium with fresh warm Kupffer Cell Maintenance Medium (MCKM250).
8. After 24 hours, replace the medium with warmed Kupffer Cell Maintenance Medium (MCKM250) and proceed with your experiment.

Frequently Asked Questions

1. Can I expand these cells?

   We do not recommend passaging the Kupffer cells, these primary cells are designed to be thawed and used one time only.

2. Why can I not use pre-warmed medium to thaw the Kupffer cells?

   Kupffer cells easily attach to the walls of the conical tube at 37°C. Using cold medium helps minimize loss of cells during the thawing process.

3. What happens if the cells are allowed to attach longer than 6 hours?

   The cells will begin to die and your attachment will be significantly lower.

4. Can I co-culture the Kupffer cells with hepatocytes?

   Yes, the recommended ratio for normal conditions is 8 hepatocytes to 1 Kupffer cell. For inflammatory conditions, the recommended ratio is 2 hepatocytes to 1 Kupffer cell. Kupffer cells are plated first. For best results, hepatocytes are seeded 24 hours after plating Kupffer cells.

Example Images