Human Kupffer Cell Protocols

It is important to read and understand the following instructions prior to use. Improper handling may adversely affect cell quality and performance.

Recommended supplies and reagents

Kupffer Cell (KC) Culture Media: Corning RPMI 1640 (cat. # 10-040-CV) + 10% FBS (such as Gemini Biologicals cat. # 100-106) + 1% Penicillin/Streptomycin solution (such as Gibco cat. # 15140-122)

Culture Vessels: Tissue culture treated plastic ware or collagen type 1 coated plastic ware

Thawing Procedure

Note: (Keep cells on ice and cold until CENTRIFUGE PROCEDURE)

1. In Biological Safety Cabinet (BSC): Place 9 mL of cold KC Culture Media in a 15 mL conical tube and keep on ice. Keep the rest of KC Culture Media at room temperature for steps in CENTRIFUGE PROCEDURE and PLATING PROCEDURE.
2. Hold cryovial(s) containing Kupffer cells 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 15 mL conical tube of cold KC Culture Media. Scale volume up for additional vials (ex. 5 vials into 50 mL). 15 mL conical tubes are recommended for individual vial thaw to achieve better post-thaw yield and viability.
5. In BSC: Remove 1 mL of the cell suspension from the 15 mL tube and use it to rinse the cryovial(s) to capture residual cells; return the 1 mL rinse to the 15 mL tube and recap tube.
6. In BSC: Gently invert the 15 mL conical tube 5-6 times to mix well.

**** Please note that human Kupffer cells do not proliferate in culture and cannot be passaged; therefore, the appropriate number of vials must be thawed to obtain the desired number of cells.****

Centrifuge Procedure

1. Centrifuge cells at 500 x g for 5 minutes.
2. In BSC: After centrifugation, gently aspirate supernatant then re-suspend pellet immediately in appropriate volume of fresh cold KC Culture Media for cell counting.

Plating Procedure

1. Determine cell number and viability using lab standard methods and procedures.
2. In BSC: Add additional KC culture Media to bring the cells to a concentration of 0.2 – 0.4 $X10^6$ 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 @ 5% CO_2.
5. In BSC: After 4-6 hours, carefully aspirate the media and replace with an equal volume of fresh warm KC Culture Media.

Cell Culture Maintenance Procedure:

1. Adult human Kupffer cells can be maintained up to 7 days.
2. In BSC: Aspirate and replace KC Culture Media every day or as required by the experiment.
3. Continue this schedule until the conclusion of the experiment.

63-0286.00

Saving Lives. Restoring Health. Giving Hope.