Thawing, Counting, and Re-suspension of Cryopreserved Suspension Hepatocytes

Hepatocyte Reagents and Materials	**Order Information**	**Catalog Number**
IVAL Suspension Hepatocytes	IVAL	see PCS
Thawing Medium		
• UCRM™ - Universal Cryopreservation Recovery Medium, 50 mL	IVAL	81015
Suspension Medium		
• HQM™ - Hepatocyte Incubation Medium, with supplements, 50 mL / 500 mL	IVAL	81039/81040

Laboratory Tools for Thawing, Counting and Re-suspension of Hepatocytes

Prior to thawing hepatocytes, ensure the Biological Safety Cabinet (BSC) is equipped with the following:

- 37°C UCRM™
- Ice bucket containing ice
- 4°C HQM™
- Serological, P1000 and P200 pipettes and appropriate sterile tips
- Multichannel pipettes and appropriate sterile tips may be used for small well-formats
- Waste container
- Sterile microcentrifuge tubes
- Trypan Blue and DPBS or Medium
- Hemocytometer

Thawing Procedure

1. Warm the 50 mL centrifuge tube of UCRM™ in a 37°C water bath for 30 minutes. Following the 30-minute incubation at 37°C, transfer UCRM™ into the BSC and remove sealing film. Place the tube of HQM™ in the ice bucket containing ice in the BSC.
2. Quickly transfer a vial of cryopreserved hepatocytes from the liquid nitrogen storage dewar into the 37°C water bath. Immerse the vial so that the contents are below the waterline and shake gently until the vial is almost completely thawed. The thawing process is approximately 2 minutes. Keep the vial in the water bath while thawing. Removing hepatocytes from the water bath prematurely will cause the hepatocytes to re-freeze and severely reduce viability. As the last ice crystal is about to disappear, remove the vial from the water bath, spray or wipe the vial with 70% alcohol, and place the vial on ice inside the BSC. It is important to place the vial on ice until you are ready to pour the contents into UCRM™ as the cryopreservant is toxic to the hepatocytes at temperatures above 12°C.
3. Pour the thawed hepatocytes into the UCRM™ medium. Use the P1000 with a sterile tip to rinse the vial 3 times with 700 µL of the UCRM™ to ensure all the hepatocytes have been transferred from the vial into UCRM™.
4. Tighten the cap of the UCRM™ 50 mL conical, invert the tube gently a few times, and centrifuge at:
    a. 10 minutes at 100 x g at room temperature for human and monkey hepatocytes
    b. 5 minutes at 100 x g at room temperature for dog and rat hepatocytes
    c. 5 minutes at 50 x g at room temperature for mouse hepatocytes
5. After centrifugation, be careful to keep the pellet intact. Spray or wipe the outside of the conical tube with 70% alcohol, and without inverting the tube, return it to the BSC.
6. Pour out the entire supernatant into a waste container in one motion. It is important to pour in one motion and not revert the tube until all the contents are poured out. Reversion will disturb the pellet and require re-centrifugation, which will cause cell damage.

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7. Add approximately 4 mL of 4°C HQM™ down the side of the UCRM™ conical tube. Gently rock the cell pellet with the media until the cell pellet is dispersed and the cells are re-suspended. Do not vortex or shake vigorously. Keep the cell suspension on ice.

Counting Procedure

8. Count the hepatocytes using the Trypan Blue exclusion method. Prepare hepatocytes for counting (25 µL DPBS or Medium + 25 µL Trypan Blue + 50 µL cell suspension) and mix the contents by inverting the tube. Load the hemocytometer by applying 10 µL from the microcentrifuge tube. Scan the hemocytometer through the microscope to ensure an even distribution of hepatocytes. Count using 10X magnification and complete the following calculations. 25 µL of DPBS or Medium and 25 µL Trypan Blue and 50 µL of cell suspension creates a dilution factor of 2 for the calculation below.
9. Hepatocytes in suspension adjusted to the 1.0 density are ready to proceed according to specific experimental guidelines. Hepatocytes are prepared at a cell density of 1.0, and incubated at a cell density of 0.5 million hepatocytes/mL in a non-coated 24 well plate with the appropriate substrates.

Calculations

Cell Count Information

Cell Count Dilution	_____	Viable Cells	_____
# of Quadrants	_____	Non-Viable Cells	_____
# of Vials	_____	Total Cells	_____

Viability

_____(Viable Cells) / _____ (Total Cells) x 100 = _____%

Cell Density

_____ (Viable Cells) / _____ (# of Quadrants) x 10,000 x _____ (Dilution) = _____ x 106 cells/ mL.

Viable Yield

_____10^6 cells/mL x _____mL (Cell Suspension Volume) = _____x 106 cells

Total Volume from Species Specific Cell Density

_____x 10^6 cell (Viable Yield) / _____ x 106 cells/mL (Optimal Cell Density) = _____ (mL) Total Volume

Adjust the Cell Concentration Volume

_____ (mL) Total Volume - _____ (mL) Cell Suspension Volume = _____ (mL) Volume of Media to add

Lot Specific Information

To inquire about our products and services or for technical questions please contact:
In Vitro ADMET Laboratories by phone at +1 (866) 458-1094 or +1 (410) 869-9037 or email at info@invitroadmet.com

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