Thawing, Counting, and Plating Plateable Cryopreserved Hepatocytes

Hepatocyte Reagents and Materials	Order Information	Catalog Number
IVAL Plateable Cryopreserved Hepatocytes	IVAL	see PCS
Thawing Medium		
• UCRM™ - Universal Cryopreservation Recovery Medium, 50 mL	IVAL	81015
Plating Medium		
• UPCM™ - Universal Primary Cell Plating Medium, 50 mL / 500 mL (Human, Monkey, Rat)	IVAL	81016 / 81017
• IPM-A™ - IVAL Plating Medium-A, 50 mL / 500 mL (Dog, Mouse)	IVAL	81035 / 81036
CellAffix™ Collagen-Coated Plates		
• CellAffix™ Collagen I Coated Plate, 6-well, 5/pack	APS	71004
• CellAffix™ Collagen I Coated Plate, 12-well, 5/pack	APS	71005
• CellAffix™ Collagen I Coated Plate, 24-well, 5/pack	APS	71006
• CellAffix™ Collagen I Coated Plate, 48-well, 5/pack	APS	71007
• CellAffix™ Collagen I Coated Plate, 96-well, 5/pack	APS	71008
• CellAffix™ Collagen I Coated Plate, 384-well, 5/pack	APS	71009

Laboratory Tools for Thawing, Counting, and Plating 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 UPCM™ or IPM-A
- 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 UPCM™ or IPM-A™ 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™.

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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.
7. Add approximately 4 mL of 4°C UPCM™ or IPM-A™ down the side of the tube containing the cell pellet. 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. 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 are adjusted to the 0.7 million cells per mL for human, monkey, dog, and rat. Mouse hepatocytes are adjusted to 0.4 million cells per mL.

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 10^6 cells/ mL.

Viable Yield

_____10^6 cells/mL x _____mL (Cell Suspension Volume) = _____x 10^6 cells

Total Volume from Species Specific Cell Density

_____x 10^6 cell (Viable Yield) / _____ x 10^6 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

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Plating Procedure

10. Add UPCM™/ IPM-A™ to the cell suspension according to the calculated Cell Concentration Volume. Pipette the appropriate volume of hepatocyte suspension to each well according to the table below. Re-suspend the cell suspension prior to and during plating to ensure a consistent cell concentration. Assess the seeding density at the microscope as needed.

Well Format	Hepatocytes per Well			Hepatocytes per Plate (x10^6)			Overlay and Maintenance	
	Volume / Well	Human, Monkey, Dog, Rat	Mouse	Volume / Plate	Human, Monkey, Dog, Rat	Mouse	Volume / Well	Volume / Plate
6-well	2.4 mL	1.68 Million	960,000	14.4 mL	10.1	5.8	2.0 mL	12.0 mL
12-well	950 µl	665,000	380,000	11.4 mL	8.0	4.6	1.0 mL	12.0 mL
24-well	500 µl	350,000	200,000	12.0 mL	8.4	4.8	500 µl	12.0 mL
48-well	188 µl	131,600	75,200	9.1 mL	6.3	3.6	250 µl	12.0 mL
96-well	80 µl	56,000	32,000	7.7 mL	5.4	3.1	100 µl	12.0 mL

11. Gently place 6-well, 12-well, or 24-well into a 37°C incubator maintained at 95% balanced air and 5% CO_2 immediately after seeding and shake North-South, East-West – 3 shakes per direction. Repeat to ensure an even cell distribution.

    Note: When seeding a 48-well or 96-well plate format, leave the plate under the BSC untouched for 30 minutes for cells to attach. Do not shake or touch the plate after adding the hepatocytes. After 30 minutes, transfer the plate, without shaking, to a 37°C incubator maintained at 95% balanced air and 5% CO_2 and incubate for an additional 4 hours. The 48-well or 96-well does not need to be shaken when placing the plate into the incubator.

12. In general, the hepatocytes can be used after 4-6 hours of attachment for experimentation (e.g. change medium to that containing test articles for cytotoxicity studies).
13. For the remainder of the experiment, continue to change medium according to specific experimental guidelines. Refer to the table above for recommended maintenance volumes.

Lot Specific Information

To inquire about our products and services or for technical questions please contact:
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