PROTOCOL – THAWING CRYOSTAX® CRYOPLATEABLE HEPATOCYTES

The following procedure should be carried out in a biosafety containment hood to reduce the risk of contamination and minimize contact with potentially biohazardous material.

Product No.	Legacy PN	Description	Size
F00995-CXP	HP1500.HP	Female CryostaX Human Individual Cryoplateable Hepatocytes	5 Million
M00995-CXP	HP1000.HP	Male CryostaX Human Individual Cryoplateable Hepatocytes	5 Million
X008000-CXP	HPCH5+	Mixed Gender CryostaX Human 5-Donor Pooled Cryoplateable Hepatocytes	5 Million
X008001-CXP	HPCH10+	Mixed Gender CryostaX Human 10-Donor Pooled Cryoplateable Hepatocytes	5 Million
M00005-CXP	RPCH1000+	Male CryostaX Sprague-Dawley Rat Pooled Cryoplateable Hepatocytes	5 Million
F00005-CXP	RPCH1500+	Female CryostaX Sprague-Dawley Rat Pooled Cryoplateable Hepatocytes	5 Million
M00305-CXP	PPCH2000+	Male CryostaX Cynomolgus Monkey Pooled Cryoplateable Hepatocytes	5 Million
M00305-CXP-C	PPCH2000+	Male CryostaX Cynomolgus Monkey Pooled Cryoplateable Hepatocytes, CITES	5 Million
M005042-CXP	MPCH1000+	Male CryostaX CD-1 Mouse Pooled Cryoplateable Hepatocytes	2 Million
F005042-CXP	MPCH1000+	Female CryostaX CD-1 Mouse Pooled Cryoplateable Hepatocytes	2 Million

Stepwise Procedure for Thawing Kits (K8000 and K8800)

This procedure describes the steps required for the isolation of hepatocytes using BioIVT's CryostaX Hepatocyte Thawing Kits (previously known as XenoTech OptiThaw Hepatocyte Thawing Kits).

Recommended Materials:

- CryostaX Hepatocyte Thawing Medium (Previously called XenoTech OptiThaw Hepatocyte Thawing Medium)
- CryostaX Counting Solution: 50 µL Trypan Blue and 400 µL of 1xPBS
- Cryostax Hepatocyte Plating Medium (Previously called XenoTech OptiPlate Hepatocyte Plating Medium)
- Cryostax Hepatocyte Culture Medium (Previously called XenoTech OptiCulture Hepatocyte Culture Medium)

Procedure:

1) Warm the CryostaX thawing medium to 37 ± 1°C in water bath or heated incubator before use (Warming the medium typically takes ~15-20 minutes.)
2) Remove the cryotube from the LN2 storage unit and immediately dispense it into the pre-warmed CryostaX Thawing Hepatocyte Medium. Do not thaw CryostaX in a water bath. Once the frozen pellets are transferred to CryostaX Thawing Medium, gently invert the tube until all the pellets have fully melted.
3) Centrifuge at 100 x g for 5 minutes. A specific temperature is not required. Aspirate and discard the supernatant fluid without disturbing the cell pellet.
4) DO NOT VORTEX. Resuspend the cell pellet with 2.0-3.0 mL of K8200 CryostaX Hepatocyte Plating Medium by gently swirling the medium in the tube.
5) Remove 50 µL of the homogenous cell suspension and dispense the 50 µL aliquot into the counting tube. Mix gently.
6) Assess cell viability by placing an aliquot from the counting tube on a hemacytometer and counting the dead (blue) cells and viable cell number.
7) Measure the volume of the cell suspension and dilute with CryostaX Hepatocyte Plating Medium to achieve the desired concentration of hepatocytes. The table below shows a range of recommended seeding densities for each species. See the lot specific datasheet for lost specific recommended seeding density for specific plate formats.

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8) Add the recommended volume of diluted cells to each culture well. The table below provides recommended seeding volumes for the various species and plate formats. For efficient attachment of the cryoplateable cells, type I collagen coated culture vesicles are needed.

Species	6-Well Format		12-Well Format		24-Well Format		48-Well Format		96-Well Format*	
	Recommended Seeding Density (million cells/mL)	Recommended Seeding/Feeding Volume Per Well	Recommended Seeding Density (million cells/mL)	Recommended Seeding/Feeding Volume Per Well	Recommended Seeding Density (million cells/mL)	Recommended Seeding/Feeding Volume Per Well	Recommended Seeding Density (million cells/mL)	Recommended Seeding/Feeding Volume Per Well	Recommended Seeding Density (million cells/mL)	Recommended Seeding/Feeding Volume Per Well
Human	1.0 - 1.6	1.7 mL	1.0 - 1.6	650 µL	1.0 - 1.6	330 µL	0.75	200 µL	0.75	75 µL
Rat	1.2 - 1.4	1.7 mL	1.2 - 1.4	650 µL	1.2 - 1.4	330 µL	1.2 - 1.4	150 µL	0.6 - 0.7	75 µL
Monkey	1.4 - 2.2	1.7 mL	1.4 - 2.2	650 µL	1.4 - 2.2	330 µL	1.4 - 2.2	150 µL	1.4 - 2.2	50 µL
Mouse	0.4 - 0.6	1.7 mL	0.4 - 0.6	650 µL	0.4 - 0.6	330 µL	0.4 - 0.6	150 µL	0.4 - 0.6	50 µL

** Do not swirl 96-well plates to distribute cells.*

9) Place the seeded culture vessel in the 37°C humidified CO_2 incubator and swirl in a figure 8 pattern to evenly distribute the hepatocytes in the well. *Do Not Swirl 96-well Plates*
10) While the cells are in the attachment phase, supplement CryostaX Hepatocyte Culture Medium with the antibiotics provided with the medium.
11) Allow cells to attach for 4 hours. Some cells will have flattened during that time and some will be still spherical but firmly attached to the type I collagen coated surface.
12) After cell attachment, swirl the culture vessel, to suspend the unattached cells, and aspirate the medium containing non-attached cells.
13) Add the appropriate volume of CryostaX Hepatocyte Culture Medium, chilled to 2° - 8° C, with or without CryostaX Hepatocyte Matrix Medium, to each well and return to incubator.
    a. CryostaX Hepatocyte Matrix is used for sandwich hepatocyte cultures. CryostaX Hepatocyte Matrix is diluted to 0.25 mg/mL with CryostaX Hepatocyte Culture Medium.
    b. Cold medium needs to be used to prevent polymerization of the matrix before placing on the cells.
    c. To achieve maximum confluency when plating cryoplateable rat hepatocytes, DO NOT include CryostaX Hepatocyte Matrix during the first medium change after the attachment step. The Matrix overlay should be performed 18-24 hours post plating.
14) Every 24 hours, the media should be aspirated and replaced with 37°C pre-warmed CyrostaX Hepatocyte Culture Medium.
15) Dosing with compound can begin after the hepatocytes have been in culture for 18-48 hours. Cultures typically remain viable for more than 5 days.

Related Products:

Product No.	Description	Size
K8000	CryostaX Non-Rodent Thawing Hepatocyte Kit, 47 mL, including two counting tubes	Kit
K8200	CryostaX Hepatocyte Plating Medium	40 mL
K8600/K8650	Cryostax Hepatocyte Matrix Medium	0.5 mL/1.5 mL
K8300	CryostaX Hepatocyte Culture Medium	100 mL
K8400	CryostaX Hepatocyte Incubation Medium	100 mL
K8800	CryostaX Rodent Hepatocyte Thawing Kit, 47 mL, including two thawing tubes	Kit

Caution: *This product is being sold for research and/or manufacturing purposes only. The biological samples supplied by BioIVT, or any material isolated from the samples, are for in-vitro research use only and are not to be used as a source of material for clinical therapies. Human material may be used in vivo in animals. The user assumes all responsibility for its usage and disposal, in accordance with all regulations.*

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CryostaX® Hepatocytes Sample Preparation Worksheet

This worksheet may be used to record information during the preparation of your hepatocyte sample. Prepare additional copies of this sheet as needed.

Hepatocyte Sample Identification

# Vials Thawed	
Sample ID (species/lot number)	**1.5 mL**

Date of hepatocyte isolation: _____

Trypan Blue Cell Count Analysis

A trypan blue exclusion analysis should be performed (step 5 in the thawing protocol) following re-suspension of the initial cell pellet.

Cells Counted		% Viability [A/(A+B)] x 100	Dilution factor[1]	Hemacytometer Factor[2]	Volume of sample[3]	Number of viable hepatocytes[4]	Final cell concentration[5]
Live	Dead						
A	B		C	D	E		
				10,000			
				10,000			

1. The dilution factor will equal 10 if a 50 µL aliquot of the cell suspension was dispensed into a counting tube for subsequent counting. If an alternate means of dilution was used, a different dilution factor should be calculated.
2. The hemacytometer factor will typically equal 10,000. For more information consult your hemacytometer manufacturer.
3. Volume of the sample indicates the total volume of the cell suspension from which the counting aliquot was removed.
4. The number of viable hepatocytes may be calculated from the following equation:

(A/Quadrants) X C X D X E where "quadrants" equals the number of quadrants counted on the hemacytometer.

5. The desired concentration should be determined based on the specific requirements of your experimental design.

Sample Dilution

Use the following table to calculate the final volume needed to reach the desired cell concentration.

# of viable hepatocytes (determined above)	Desired cell Concentration for use	Final volume	Volume of media to add to reach desired concentration
F	G	H	I

H = F/G I = H – Volume of Sample

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Tips for Working with Hepatocytes

• The frozen CryostaX hepatocyte pellets should be transferred directly into the CryostaX Thawing tube. Do not thaw the CryostaX hepatocyte vial in a heated water bath.

• When aspirating supernatant, keep tip of the aspirator at the highest level of media to ensure any cell debris is removed before reaching the viable cell pellets.

• BioIVT does not recommend pouring off CryostaX thaw medium supernatant, due to the high risk of losing the viable cell pellet during the pour process.

• Never vortex or vigorously resuspend the hepatocytes. A gentle rocking motion is recommended.

• We recommend performing two Trypan blue counts after centrifugation for verification of yield and viability.

• One CryostaX Hepatocyte Thawing kit can be used to thaw up to 3 vials at once.

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