PROTOCOL – THAWING CRYOSTAX® CRYOSUSPENSION 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 XenoTech Product No.	Description	Size
F00995-CX	HP1500.HP	Female CryostaX Human Individual Cryosuspension Hepatocytes	5 Million
M00995-CX	HP1000.HP	Male CryostaX Human Individual Cryosuspension Hepatocytes	5 Million
X008000-CX	HPCH20-50	Mixed Gender CryostaX Human 20-Donor Pooled Cryosuspension Hepatocytes	5 Million
X008001-CX	HPCH10	Mixed Gender CryostaX Human 10-Donor Pooled Cryosuspension Hepatocytes	5 Million
M00005-CX	RPCH1000	Male CryostaX Sprague-Dawley Rat Pooled Cryosuspension Hepatocytes	5 Million
F00005-CX	RPCH1500	Female CryostaX Sprague-Dawley Rat Pooled Cryosuspension Hepatocytes	5 Million
M00205-CX	DPCH1000	Male CryostaX Beagle Dog Pooled Cryosuspension Hepatocytes	5 Million
F00205-CX	DPCH1500	Female CryostaX Beagle Dog Pooled Cryosuspension Hepatocytes	5 Million
M00305-CX	PPCH2000	Male CryostaX Cynomolgus Monkey Pooled Cryosuspension Hepatocytes	5 Million
M00305-CX-C	PPCH2000	Male CryostaX Cynomolgus Monkey Pooled Cryosuspension Hepatocytes, CITES	5 Million
M005042-CX	MPCH1000	Male CryostaX CD-1 Mouse Pooled Cryosuspension Hepatocytes	2 Million
F005042-CX	MPCH1500	Female CryostaX CD-1 Mouse Pooled Cryosuspension Hepatocytes	2 Million
M00615-CX	ZPCH1000	Male CryostaX Gottingen Minipig Pooled Cryosuspension Hepatocytes	5 Million
F00405-CX	LPCH1500	Female CryostaX New Zealand Rabbit Pooled Cryosuspension Hepatocytes	5 Million

Stepwise Procedure for Thawing CryostaX Hepatocytes (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 Hepatocyte Incubation Medium (Previously called XenoTech OptiIncubate Hepatocyte medium)
- CryostaX Counting Solution: 50 µL Trypan Blue and 400 µL of 1xPBS

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 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 K8400 CryostaX hepatocyte incubation 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.

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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 in CryostaX hepatocyte incubation medium to achieve the desired concentration of hepatocytes.

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
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.*

Tips for Working with Hepatocytes

• The frozen CryostaX hepatocyte pellets should be transferred directly into the CryostaX Hepatocyte 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 pellet.

• 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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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 CryostaX 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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