mosaic cell sciences a division of Organovo

Certificate of Analysis

Product Name: Human Hepatocytes - cryopreserved
Item Number: 120803-03

Lot Number: HL240015HC
Grade: Plateable

Storage Condition

Short Term (<24 hr): Dry ice (-78.5°C)	Long Term (> 24 hr): Vapor phase liquid nitrogen

Donor Demographics

Age:	**59**
Ethnicity:	**White**
Gender:	**Male**
BMI:	**33.1**
Previous Medical History:	**Hypertension**
Regular Medications:	**Unknown blood pressure medication**
Disease Status:	**Healthy**
HbA1c	**5.4%**
Cause of Death:	**Stroke**
History of smoking:	**No**
Alcohol use:	**1-2 per week**
Drug use:	**None**

Serology

Anti-CMV:	Negative
EBV:	Negative
Anti-HCV:	Negative
Anti-HBcAb:	Negative
HBsAg:	Negative
HBsAb (optional):	Not done
Anti-HIV I/II:	Negative
Anti-HTLV I/II (optional):	Not done
Syphilis:	Negative

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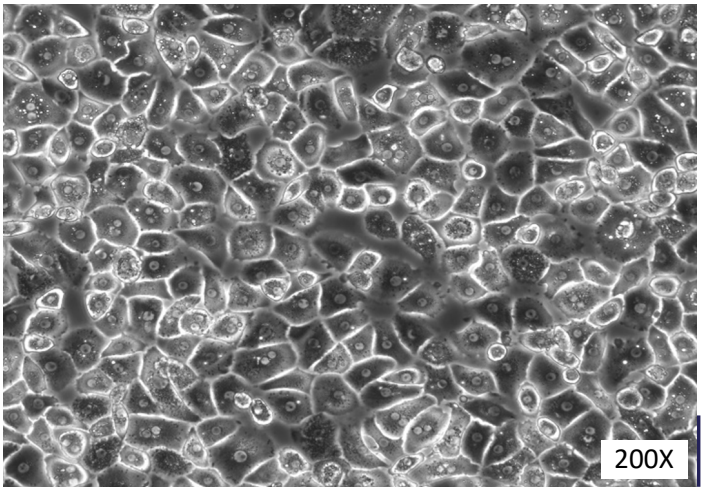
Quality Control Results	
Appearance:	Tannish liquid when thawed
Post-Thaw Yield:	>7x10^6 cells/vial
Post-Thaw Viability (Trypan Blue):	>80%
Culture Morphology:	See figure 1

HLA				
Class I	A	2	A	29
	B	13	B	44
	BW4	Positive	BW6	Negative
	C	06	C	16
Class I	DR	7	DR	7
	DR51	N-Negative	DR51	N-Negative
	DR52	N-Negative	DR52	N-Negative
	DQB1	2	DQB1	2
	DQA1	02	DQA1	02
	DPB1	11:01	DPB1	11:01
	DPA1	02	DPA1	02
	DR53	4*01	DR53	N-Negative

Figure 1. Culture Morphology

24 hour Post-thaw



Thawing and Culturing Instructions for Cryopreserved Human Hepatocytes

Universal precautions and aseptic technique should be used at all times when handling human cells

**** Please note that human hepatocytes 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. ****

RECOMMENDED SUPPLIES AND REAGENTS

Thawing Media: Hepatocyte thaw media (Life Technologies cat. # CM7500)
Plating Media: Williams' Medium E + 1% L-glutamine + 1% Pen/Strep + 5% FBS + 10-7M Insulin + 10-7M Dexamethasone + 10mM HEPES
or the following may be used,
DMEM + 1% Sodium pyruvate + 1% ITS + 1% Pen/Strep + 5% FBS + 10-7M Dexamethasone + 10mM HEPES
Maintenance Media: Williams' Medium E + 1% ITS + 10-7M Dexamethasone + 1% Pen / Strep + 10mM HEPES
Culture Vessels: Collagen type I coated culture vessels

THAWING PROCEDURE (up to 3 vials containing est. 12.0 x 10^6 cells/vial)

1. Warm Thawing Media to room temperature; clean exterior of tube with 70% ethanol before use
2. Place warmed Thawing Media in BSC and uncap
3. Hold cryovial(s) in a 370C water bath to thaw without submerging the cap in water (hold until only a sliver of ice remains, approximately 1 ½ -2 minutes)
4. Remove from water bath and clean exterior of vial(s) with 70% ethanol before placing into BSC
5. Transfer entire contents of up to 3 cryovial(s) into one 50 mL conical tube of Thawing Media
6. Remove 1.0 mL of the cell suspension from the 50 mL tube and use it to rinse the cryovial(s) to capture residual cells; return the 1.0 mL rinse to the 50 mL tube and recap tube
7. Gently invert the 50 mL conical tube 5-6 times to mix well

CENTRIFUGE PROCEDURE

1. Centrifuge cells at 100 x g for 10 minutes at room temperature
2. After centrifugation, aspirate supernatant then re-suspend pellet in 3-5 mL of fresh Plating Media for each vial thawed

PLATING PROCEDURE

1. Determine viable cells using lab standard methods and procedures
2. Add additional Plating Media to get the desired cell concentration
3. Dispense the desired cell number into the culture vessel and swirl gently to distribute
4. Place culture vessels in humidified 37℃ incubator @ 5% CO2
5. After 4 - 12 hours, carefully aspirate the media and replace with an equal volume of Maintenance Media

CELL CULTURE MAINTANENCE PROCEDURE:

1. Aspirate and replace Maintenance Media every day or as required by the experiment
2. Continue this schedule until the conclusion of the experiment.

Results above determined by following Mosaic Cell Sciences' hepatocyte care and handling protocols.

Ken Dorko, Sr Director Cell Processing

09-MAY-2024

Date