LifeNet Health
LifeSciences Division

CERTIFICATE OF ANALYSIS

CELL TYPE:	Primary Human Hepatocytes	Lot No:	2019300-01
Storage Condition:	Cryopreserved in Vapor Phase Liquid Nitrogen (< -150°C)		

DONOR DEMOGRAPHICS

Age	Sex	Race	BMI	Tobacco Use†	Alcohol Use†	Drug Use†	Medication Use†	Cause of Death
46	Female	African American	35	N/A	See below	N/A	See below	Anoxia

†ADDITIONAL TOBACCO, ALCOHOL, DRUG, AND MEDICATION HISTORY

Alcohol – Wine 3-4 glasses, 2-3 times a week for 3 years; Medication – NOK did not know names of any medications

LIVER FUNCTION DATA – PREMORTEM LAB VALUES				SEROLOGY TEST RESULT
Total Bili: 0.4	AST: 83	PT: 9.5	Total Protein: 6.5	Negative for HIV-1 and HIV-2
Direct Bili: N/A	ALT: 43	PTT: N/A	Alk Phos: 147	Negative for Hepatitis-B and Hepatitis-C

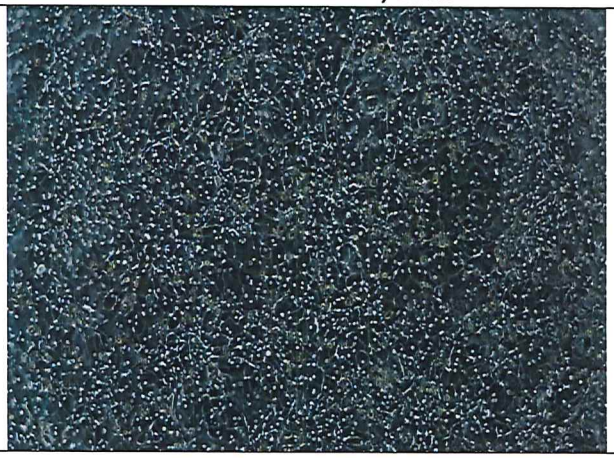
POST THAW VIABILITY		MONOLAYER ASSESSMENT		
Average Viability	Average Viable Cells/Vial	Optimal Seeding Density	Initial Attachment Efficiency	Confluency After 120 hrs in Culture
89%	7.1×10^6	0.9×10^6	95%	95%

Thawing Instructions	Follow normal thawing instructions in technical bulletin.

PHOTOMICROGRAPHS

24 hrs Post Thaw, 100X

120 hrs Post Thaw, 100x



Signature: Ryan Bart Date: 02 October 2020

Print Name: Ryan Barture Title: Life Sciences Technician

Authorization was obtained from the donor or the donor's legal next of kin for use of the tissue and its derivatives for research purposes.
Caution: The user should treat all human cells as potential pathogens. Wear protective clothing and eyewear. Practice appropriate disposal techniques for potentially pathogenic or biohazardous materials. For research use only. Not intended for human or animal therapeutic or diagnostic use.

For more information: **cells_tissues@lifenethealth.org**

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CERTIFICATE OF ANALYSIS - HISTOLOGY

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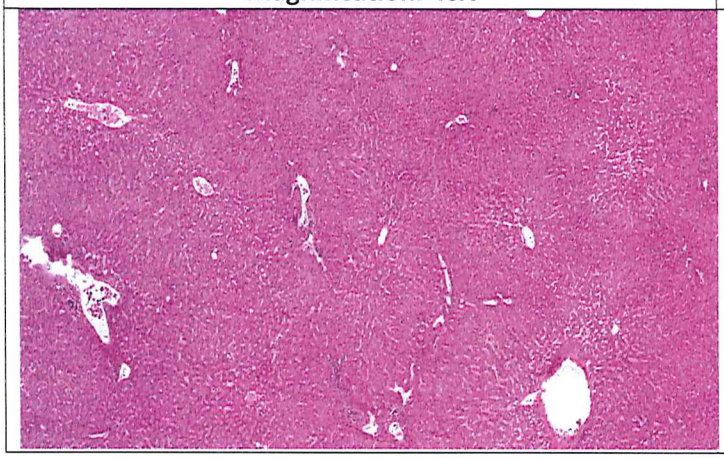
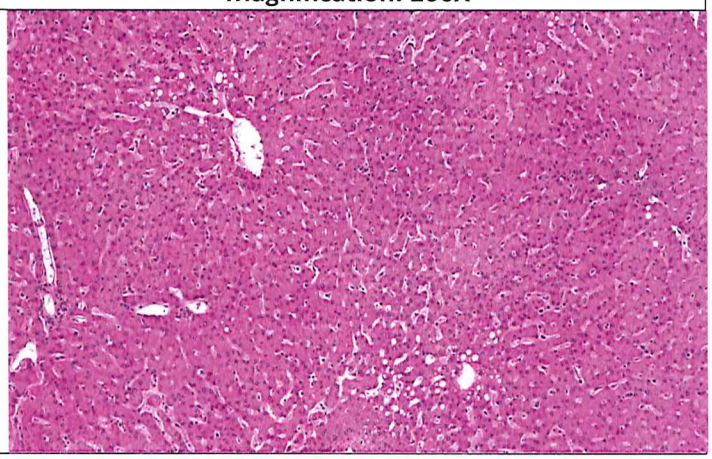
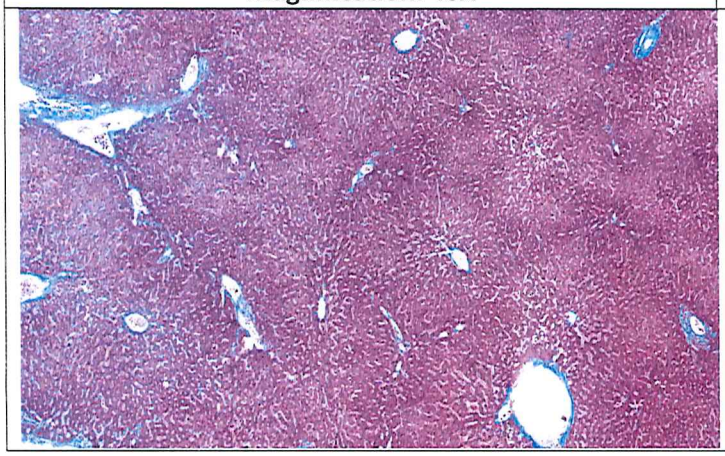
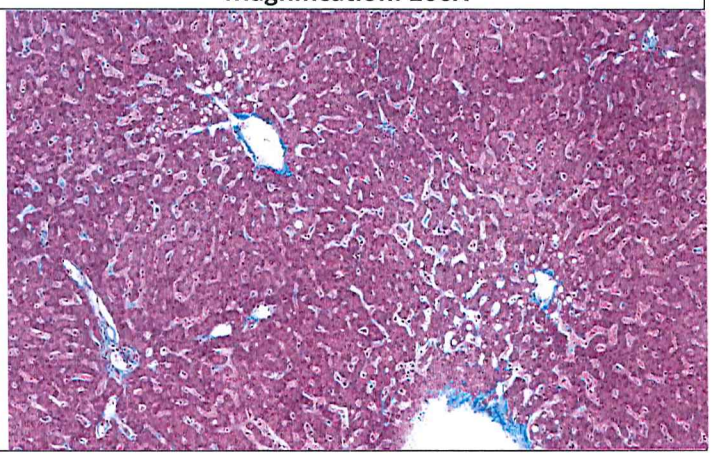
H&E - REPRESENTATIVE IMAGES

Magnification: 40X	Magnification: 100X
	

TRICHROME - REPRESENTATIVE IMAGES

Magnification: 40X	Magnification: 100X
	

HISTOPATHOLOGICAL SCORING

NAS Score*	Steatosis Score	Lobular Inflammation Score	Hepatocyte Ballooning Score	Fibrosis Stage
1	1	0	0	0

*NAS scores 0-2 are not considered diagnostic of NASH, scores of 3-4 can be considered diagnostic of NASH with high interobserver variability, and scores of 5-8 are diagnostic of NASH.

Additional Information/Comments:
Mild steatosis (~15-20% of hepatocytes). No excess inflammation or fibrosis.

Signature: Kristina Wolf Date: 19 Oct 2020

Print Name: Kristina Wolf Title: Scientist, R+D, Life Sciences

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CERTIFICATE OF ANALYSIS – CYP PROFILE

CELL TYPE:	Primary Human Hepatocytes	Lot No:	2019300-01
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ENZYME SPECIFIC ACTIVITY (pmol/min/million cells)

PHEN	COUM	BUP	AMO	DICL	MEPH	DEX	CZX	TEST	MID	7-EC	7-HC	7-HC
1A2	2A6	2B6	2C8	2C9	2C19	2D6	2E1	3A4	3A4	PHI	PHII-G	PHII-S
27.8	24.9	79.7	338	766	13.9	77.2	140	981	319	10.3	225	92.7

METHOD SUMMARY

Cryopreserved human hepatocytes were thawed with HHTM and resuspended at a density of 1.0 x 10^6 cells/mL in HHCM. Cells were added to 24-well ultra-low attachment plates and placed on an orbital shaker in a humidified 37°C incubator for 10 min. After removal from the incubator, 2x substrate solutions (37°C) were added to the corresponding wells (n=3 wells per substrate), resulting in a final 1x substrate concentration (see table below). The plates were returned to the orbital shaker in the incubator and the reactions allowed to proceed for the amount of time specified (see table below). Enzyme activity was calculated by dividing the amount of metabolite produced by the reaction time and number of cells in the reaction.

Enzyme	Substrate	Substrate Concentration (µM)	Incubation Time (min)	Metabolite
CYP1A2	Phenacetin	100	15	Acetaminophen
CYP2A6	Coumarin	50	15	7-Hydroxycoumarin
CYP2B6	Bupropion	500	15	Hydroxybupropion
CYP2C8	Amodiaquine	20	30	*N*-Desethylamodiaquine
CYP2C9	Diclofenac	25	15	4'-Hydroxydiclofenac
CYP2C19	(*S*)-Mephenytoin	250	30	4'-Hydroxymephenytoin
CYP2D6	Dextromethorphan	15	15	Dextrorphan
CYP2E1	Chlorzoxazone	250	15	6-Hydroxychlorzoxazone
CYP3A4/5	Testosterone	200	15	6β-Hydroxytestosterone
CYP3A4/5	Midazolam	20	15	1'-Hydroxymidazolam
Phase I (CYPs)	7-Ethoxycoumarin	100	30	7-Hydroxycoumarin
Phase II (UGTs)	7-Hydroxycoumarin	100	30	7-Hydroxycoumarin Glucuronide
Phase II (SULTs)	7-Hydroxycoumarin	100	30	7-Hydroxycoumarin Sulfate

Signature: Kristina Wolf Date: 30 Sep 2020

Print Name: Kristina Wolf Title: Scientist, R+D, Life Sciences

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CERTIFICATE OF ANALYSIS - INDUCTION

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ENZYME INDUCTION

Isoform	Inducer	Control Activity (pmol/min/10^6 cells)	Induced Specific Activity (pmol/min/10^6 cells)	Induced Specific Activity Fold Induction	mRNA Fold Induction
CYP1A2	50 µM Omeprazole	11.7	300.9	25.8	43.5
CYP2B6	1000 µM Phenobarbital	0.7	2.8	4.2	8.4
CYP2B6	0.25 µM CITCO	1.6	14.0	8.5	8.1
CYP3A4	10 µM Rifampicin	26.8	149.0	5.6	11.5

METHOD SUMMARY

Cryopreserved human hepatocytes were thawed with HHTM, plated in 24-well BioCoat™ plates in HHPM, and overlaid 8-10 hrs post-plating with HHCM containing 0.25 mg/mL Matrigel®. Cultures were treated (n=3 wells per compound) with vehicle control [0.1% (v/v) DMSO] or inducers in HHCM for either 48 hrs (mRNA) or 72 hrs (specific enzyme activity). At the end of the treatment period, RNA was isolated from the induction plate for mRNA analysis. The plate for specific enzyme activity was incubated with probe substrates (see table below) for 30 min. Fold induction was calculated by dividing induced activity by vehicle control activity (specific activity) or using the $\Delta\Delta C_T$ method (mRNA expression).

Enzyme	Probe Substrate	Concentration (µM)	Probe Metabolite
CYP1A2	Phenacetin	100	Acetaminophen
CYP2B6	Bupropion	50	Hydroxybupropion
CYP3A4	Midazolam	10	1'-Hydroxymidazolam

Signature: Kristina Wolf Date: 18 Dec 2020

Print Name: Kristina Wolf Title: Scientist, R+D, Life Sciences

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