AnaBios
Early Human Insights

CERTIFICATE OF ANALYSIS
CRYOPLATEABLE HUMAN HEPATOCYTES

FOR RESEARCH USE ONLY. CAUTION: Not intended for human diagnostic or therapeutic uses. Users should treat all human cells as potential pathogens. Wear protective clothing and eyewear. Practice appropriate disposal techniques for potentially pathogenic or bio-hazardous materials.

PRODUCT INFORMATION:

Product name: Primary Human Hepatoyctes

Lot identifier: NHP0003A

Post-thaw viability of ≥ 80 %

Post-thaw confluency ≥ N/A % on day 5

Contains a minimum of 12 x 10^6 viable cells/mL

DONOR DEMOGRAPHICS:

Sex: Male Age: 67 BMI: N/A Ethnicity: N/A Diagnosis: NASH

Donor negative for: HIV, HCV, HBV, RPR

Culture negative for: Gram+, Gram-, Mycoplasm, Fungi

The cells from this lot were derived from tissue obtained from accredited institutions. Consent was obtained by these institutions from the donor or the donor's legal next of kin, for use of the tissue and its derivatives for research purposes.

Sr. VP, Research Alliances

11/07/2024

Date

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ENZYME INDUCTION ACTIVITY

ENZYME	TREATMENT	mRNA FOLD-CHANGE
CYP1A2	Omeprazole (25 μM)	
CYP2B6	Phenobarbital (2 mM)	
CYP3A4	Rifampin (10 μM)	

Hepatocytes were seeded on a 24-well plate pre-coated with collagen I, and cultured for 48 hours at 37°C in 5% CO_2 and 90% humidity. The hepatocytes were then incubated at 37°C in 5% CO_2 and 90% humidity with omeprazole (25 μM, CYP1A2), phenobarbital (2 mM, CYP2B6), rifampin (10 μM, CYP3A4) and vehicle control for 48 hours, respectively. The media were replaced every 24 hours with fresh media containing the positive inducers and vehicle. The mRNA levels of CYP1A2, CYP2B6 and CYP3A4 were determined using RT-PCR.

METABOLIC ACTIVITY

ENZYME	SUBSTRATE	CONCENTRATION (μM)	ENZYME ACTIVITY *(pmole/min/million cells)*
CYP1A2	Phenacetin	100	
CYP2A6	Coumarin	50	
CYP2B6	Bupropion	500	
CYP2C8	Amodiaquine	20	
CYP2C9	Diclofenac	25	
CYP2C19	S-mephenytoin	250	
CYP2D6	Dextromethorphan	15	
CYP2E1	Chlorzoxazone	250	
CYP3A4	Testosterone	100	
CYP3A4	Midazolam	20	
Phase I CYP	7-ethoxycoumarin	100	
SULT	7-hydroxycoumarin sulfation	100	
UGT	7-hydroxycoumarin glucuronidation	100	

Hepatocytes in suspension (0.5 million/mL) were incubated with substrate at 37°C in 5% CO_2 and 90% humidity for 30 minutes, respectively. The concentrations of the metabolites were determined using LC-MS/MS methods.

TRANSPORTER ACTIVITY

TRANSPORTER	SUBSTRATE	UPTAKE ACTIVITY RATE *(pmole/min/million cells)*
OATP1B1/3	Estrone 3-sulfate	
OCT1/2	1-Methyl-4-phenylpyridinium iodide	
NCTP	Taurocholic acid	

Hepatocytes in suspension (0.5 million/mL) were incubated in substrate (10 µM) on ice and then at 37°C for 3 minutes, respectively. Hepatocytes were separated from the medium by oil-spin method. The substrate concentrations were determined by specific LC/MSMS method.

TRANSPORTER	SUBSTRATE	EXPORT ACTIVITY RATE *(pmole/min/million cells)*
BSEP	Glycocholic acid	

Hepatocytes in suspension (0.25 million/mL) were incubated with cholic acid-d_4 (10 µM) at 37°C in 5% CO_2 and 90% humidity for 60 minutes. The concentration of glycocholic acid were determined using a LC/MSMS method.