

## 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:	Lot identifier:
Post-thaw viability of ≥%	Post-thaw confluency ≥% on day 5
Contains a minimum of x 10 <sup>6</sup> viable cells/mL	
DONOR DEMOGRAPHICS:	
Sex: Age: BMI: Ethnicity:	Diagnosis:
Donor negative for: HIV, HCV, HBV, RPR	Culture negative for: Gram+, Gram-, Mycoplasm, Fungi
	ined from accredited institutions. Consent was obtained egal next of kin, for use of the tissue and its derivatives
Sr VP Research Alliances	Date



1155 Island Avenue, Suite 200
San Diego, CA 92101
<a href="https://www.anabios.com">www.anabios.com</a>
For inquiries, please contact us at <a href="mailto:info@anabios.com">info@anabios.com</a>.

## **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  $37^{\circ}$ C in 5% CO<sub>2</sub> and 90% humidity. The hepatocytes were then incubated at  $37^{\circ}$ C in 5% CO<sub>2</sub> and 90% humidity with omeprazole ( $25 \mu$ M, CYP1A2), phenobarbital ( $2 \mu$ M, CYP2B6), rifampin ( $10 \mu$ M, 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  $37^{\circ}$ C in 5% CO<sub>2</sub> 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  $\mu$ M) 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-d4 (10  $\mu$ M) at 37°C in 5% CO<sub>2</sub> and 90% humidity for 60 minutes. The concentration of glycocholic acid were determined using a LC/MSMS method.