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 Hepatocytes

Lot identifier: 1045

Post-thaw viability of ≥ 90 %

Post-thaw confluency ≥ 95 % on day 5

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

DONOR DEMOGRAPHICS:

Sex: Female Age: 2 BMI: 15.6 Ethnicity: Caucasian COD: Anoxia/Drowning

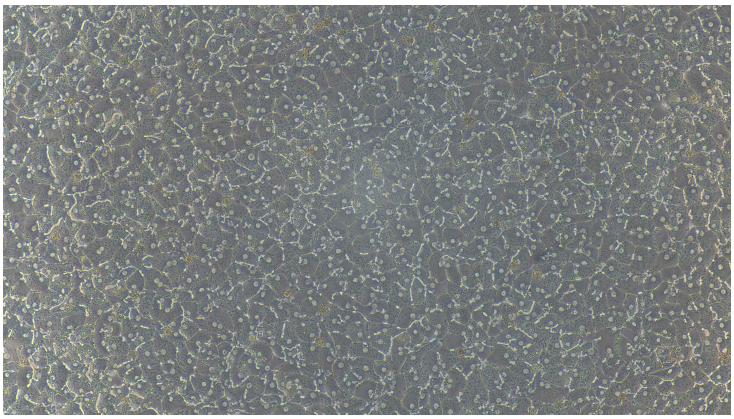
Donor negative for: HIV, HCV, HBV, RPR

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

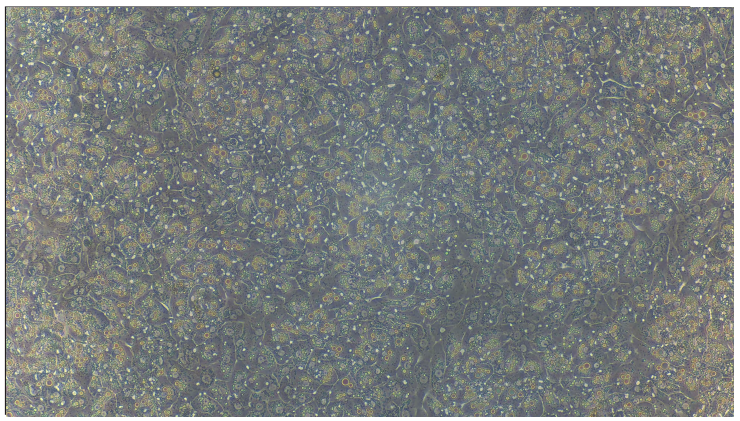
PLATING:

Recommended seeding density (million cells/mL): 0.8-1.0

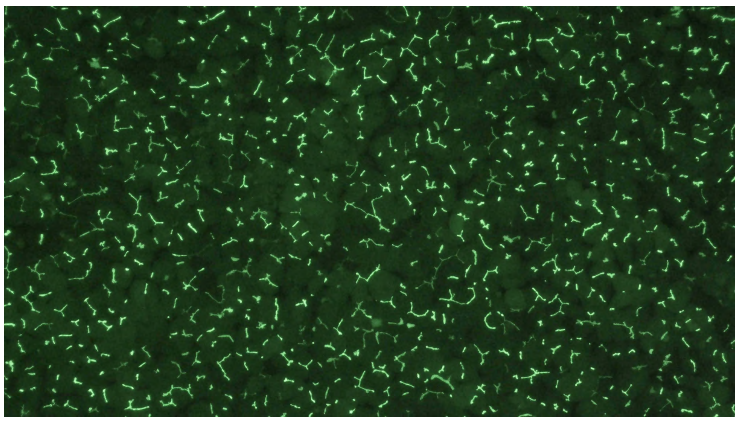
Recommended thaw medium: AnaBios Thaw Medium A



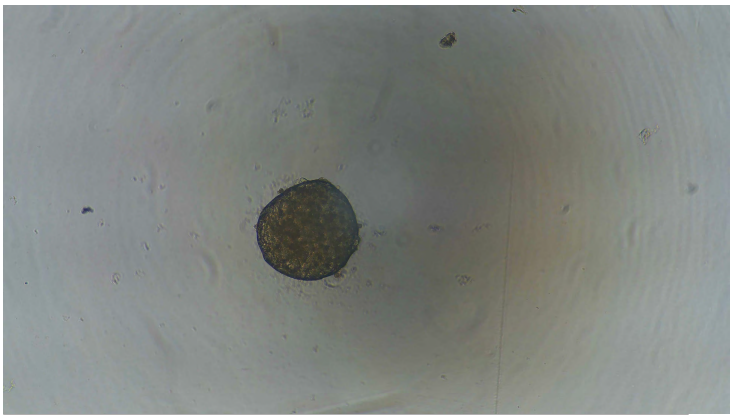
Lot 1045 5 days post-plating



Lot 1045 14 days post-plating



CDFDA Stain of Lot 1045 after 5 days post-plating



3 D spheroid formation of Lot 1045 after 11 days post-thaw.

CDFDA Stain: Carboxy-DCFDA was used to visualize the bile canaliculi formation in polarized hepatocytes.

ENZYME INDUCTION ACTIVITY

ENZYME	TREATMENT	mRNA FOLD-CHANGE
CYP1A2	Omeprazole (25 µM)	301
CYP2B6	Phenobarbital (2 mM)	47.5
CYP3A4	Rifampin (10 µM)	69.2

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	199
CYP2A6	Coumarin	50	18
CYP2B6	Bupropion	500	19.5
CYP2C8	Amodiaquine	20	331
CYP2C9	Diclofenac	25	289
CYP2C19	S-mephenytoin	250	15
CYP2D6	Dextromethorphan	15	24.5
CYP2E1	Chlorzoxazone	250	79.3
CYP3A4	Testosterone	100	62
CYP3A4	Midazolam	20	271
Phase I CYP	7-ethoxycoumarin	100	13.3
SULT	7-hydroxycoumarin sulfation	100	24.5
UGT	7-hydroxycoumarin glucuronidation	100	140
AO	Zoniporide	100	8.42

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

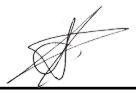
TRANSPORTER	SUBSTRATE	UPTAKE ACTIVITY RATE *(pmole/min/million cells)*
OATP1B1/3	Estrone 3-sulfate	39.2
OCT1/2	1-Methyl-4-phenylpyridinium iodide	256
NCTP	Taurocholic acid	37.4

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	27.2

Hepatocytes in suspension (0.25 million/mL) were incubated with cholic acid-d4 (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.

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.



Najah Abi-Gerges, PhD.
VP, Research & Development

8 February, 2023

Date

AnaBios
Early Human Insights

3030 Bunker Hill Street, Suite 312
San Diego, CA 92109
www.anabios.com
For inquiries, please contact us at info@anabios.com.

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3030 Bunker Hill, Suite 312
San Diego, CA 92109
Tel: 858-224-7361
Fax: 858-224-7371
www.anabios.com

Organ: Liver
AnaBios Lor Number: 1045

RESEARCH ONLY

Donor Demographic Form

Donor Information

Age:	2	Ethnicity:	Caucasian	Sex:	F
Height (cm):	94.0	Weight (kg):	13.8	BMI:	15.6
COD:	Anoxia/Drowning				

Consent for Research confirmed through visual by AnaBios Coordinator (Yes or No): Yes

Past Medical History

Respiratory Disease	No ☒	Yes ☐
Cardiac Disease	No ☒	Yes ☐
Hypertension	No ☒	Yes ☐
Neurological Disease	No ☒	Yes ☐
Cancer	No ☒	Yes ☐
Diabetes	No ☒	Yes ☐
Liver Disease	No ☒	Yes ☐
GI Disease	No ☒	Yes ☐
Kidney Disease	No ☒	Yes ☐
Urinary Tract Disease	No ☒	Yes ☐
Tobacco	No ☒	Yes ☐
Alcohol	No ☒	Yes ☐
Drug Use	No ☒	Yes ☐
Medication Home	No ☒	Yes ☐

Comments None

Serology

	(Yes/No)	Negative	Positive		(Yes/No)	Negative	Positive
Anti – HCV	**Yes**	**Negative**	-	HBs Ab	**No**	-	-
Anti – HIV 1/2	**Yes**	**Negative**	-	HBs Ag	**Yes**	**Negative**	-
Anti – HTLV 1/2	**No**	-	-	RPR/STS/VDRL	**Yes**	**Negative**	-
CMV	**Yes**	**Negative**	-	HCV NAT	**Yes**	**Negative**	-
CMV IgG	**No**	-	-	HCV RIBA	**No**	-	-
CMV IgM	**No**	-	-	HIV NAT	**Yes**	**Negative**	-
EBNA	**No**	-	-	WNV IgM	**No**	-	-
EBV IgG	**No**	-	-	HBV	**Yes**	**Negative**	-
EBV IgM	**No**	-	-	Toxo	**No**	-	-
HBc Total	**Yes**	**Negative**	-	WNV NAT	**Yes**	**Negative**	-
HBc Ab IgG	**No**	-	-	Chagas	**Yes**	**Negative**	-
HBc Ab IgM	**No**	-	-	SARS-CoV-2	**Yes**	**Negative**	-

***Authorized by*:**

Signature

18-Jan-2023
Date