CERTIFICATE OF ANALYSIS

GENTEST® HUMAN HEPATOCYTES 10-DONOR POOLED PLATEABLE

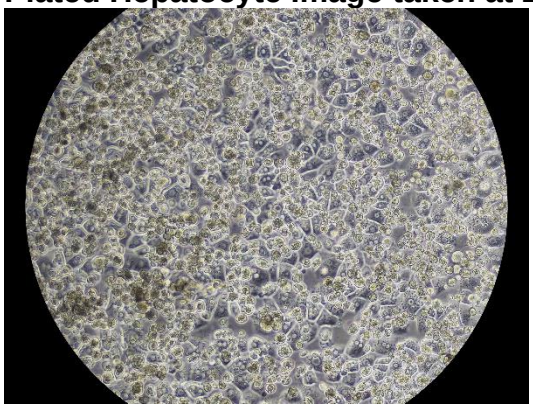
Catalog Number	4.82005	**Storage Conditions**	Store in Liquid Nitrogen
Lot Number	240604-10	**Volume**	1.5mL
Date Released	2024 August		

Post-thaw Viability and Recovery Results

Average Post-thaw Viability (%)	88
Average Viable Cells per vial (10^6 cells)	6.2
24-post plating confluency (%)	85-90%

- Hepatocytes were thawed using pre-warmed UCRM (Gentest® Cat. No. 4.81015) and centrifuged for 10 minutes at 100g at 4°C. After removing the supernatant, hepatocytes were re-suspended in 4°C UPCM-A (Gentest® Cat. No. 4.81070) and counted for viability and yield using the Trypan Blue exclusion method.

Plated Hepatocyte Image taken at 24 hours:



- Monolayer Comments: Lot 240604-10 shows good attachment efficiency and a confluency of 85-90% by 24 hours. This lot exhibits good morphology and remains intact for ≥ 5 days in culture.

Induction of CYP1A2, CYP2B6, and CYP3A4

P450 Induction	Positive Control Inducer (Concentration µM)	Substrate (Concentration µM)	Incubation Time (minutes)	Fold Induction (Activity)	Fold Induction (mRNA)
CYP1A2	Rifampicin (10)	Phenacetin (100)	60	4.6	35
CYP2B6	Omeprazole (50)	Bupropion (250)	30	5.3	9.2
CYP3A4	Phenobarbital (1000)	Testosterone (200)	30	5.6	14

- Cells were seeded at 0.08 mL per well (56,000 cells/well) in a 96-well CellAffix™ Collagen I coated plate (Gentest® Cat. No. 4.71008). After 4-6 hours, the media was changed to Williams' Medium E (supplemented with 0.1µM Dexamethasone, 0.29µg/ml L-Glutamine, 100 U/ml Penicillin, 100µg/ml Streptomycin, 10µg/ml Insulin, 5.5µg/ml Transferrin, 6.7 ng/ml Selenium, 15 mM HEPES) containing Matrigel (0.25 mg/mL) and confluence assessed. Cells were incubated overnight at 37°C with 5% CO_2.
- After 18 - 24 hours post plating, CYP induction was carried out by daily media change with induction media containing prototypical inducers for CYP1A2, 2B6 and 3A4 for 48-hours. 0.1% DMSO was used as the control for induction assays.
- On day 3, enzyme assays were performed using substrates at concentrations and incubation times described above.
- Reactions were terminated with addition of acetonitrile containing stable labeled internal standard, and the metabolite formation was analyzed by LC-MS/MS.
- Gene expression was quantified by RT-PCR.

Drug Metabolism Activity

Metabolic Pathway	Substrate	Substrate Conc. (µM)	Marker Metabolite	Metabolic Activity (pmol/million cells/minute)
CYP1A2	Phenacetin	100	Acetaminophen	34
CYP2B6	Bupropion	250	Hydroxybupropion	5.9
CYP2C8	Amodiaquine	100	Desmethylamodiaquine	350
CYP2C9	Diclofenac	100	4-OH Diclofenac	290
CYP2C19	S-Mephenytoin	250	4-OH S-Mephenytoin	5.8
CYP2D6	Dextromethorphan	25	Dextrorphan	37
CYP3A4	Midazolam	30	1-Hydroxymidazolam	34
CYP3A4	Testosterone	200	6β-Hydroxytestosterone	110
UGT	7-Hydroxycoumarin	100	7-Hydroxycoumarin Glucoronide	190
SULT	7-Hydroxycoumarin	100	7-Hydroxycoumarin Sulfate	770
FMO	Benzydamine HCl	250	Benzydamine-N-Oxide	800
AO	Carbazeran	10	4-Hydroxycarbazeran	65

- Cells were suspended at a concentration of 0.25 X 10^6 cells/mL in WEM, then 0.1 mL of cell suspension per well was added to a TC-treated 96-well plate (250,000 cells per well), and pre-incubated at 37°C, 5% CO_2 for 5 minutes. After pre-incubation time, reaction was initiated by adding 0.1 mL of 2X substrate and incubated at 37°C for 10 minutes. Reactions were terminated with addition of 0.05 mL acetonitrile containing stable labeled internal standard, and the metabolite formation was analyzed by LC-MS/MS.

Donor Information

Specimen	Gender	Age (years)	Race	Cause of Death	BMI	Social History	Medical History	Medication given during Hospitalization
311	M	75	Caucasian	ICH-Stroke	24.8	n/a	Diagnosed for HTN < 5 years ago	1/2 NS, Vasopressin, Ancef, Hydralazine, Insulin, Dexamethasone, Nimbex, Narcan, T4, Lasix, KCL, Ancef, Solumedrol, Potassium Phosphate, Atrovent, Albuterol, Vasopressin, Sodium Bicarbonate, Levophed, Levothyroxine, Fentanyl and Heparin
319	M	53	Caucasian	Head trauma-blunt injury	25.0	n/a	HTN diagnosed 2 years ago; Diabetes Type II diagnosed 2 years ago	NS, Norepinephrine, Cipro, DDAVP, Mannitol and Heparin
337A	M	58	Caucasian	Anoxia 2nd to Trauma	26.4	Alcohol use	Depression	Neosynephrine, Lasix, Vancomycin, Zosyn, Gentamicin, Cefepime, Pentobarbitol, Lamotrigine, Enoxaperine, Phenobarbitol, Lamictal, Floxacin, Miralax, Synthroid, Lovenox, Ativan
396	M	51	Caucasian	CVA 2nd to ICH	32.0	Alcohol use	HTN; undiagnosed but suspected. No treatments.	Levophed, Antihypertensives, Vasodilators
405A	M	49	Caucasian	Exsanguination 2nd to Stab	29.8	Marijuana use	n/a	Neosynephrine, Levophed, Dextrose, Antihypertensives, Vasopressin, Zosyn
377	F	57	Caucasian	CVA 2nd to ICH	26.2	Smoking and alcohol use	Skin cancer on breast-precancerous, removed and no follow-up required; HTN	Neosynephrine, NS, KCl
391	F	49	Caucasian	CVA 2nd to ICH	25.8	n/a	n/a	Levophed, Dextrose, Solumedrol, Lasix, Morphine, Heparin
383	F	57	Caucasian	CVA	27.8	Smoking and alcohol use	HTN, 18 months	n/a
HH1137	F	57	Caucasian	Anoxia	37.5	Smoking use	n/a	n/a
HH1160	F	53	Caucasian	Anoxia	32.4	Smoking, alcohol, and substance use	HTN, Diabetes	n/a

HAZARD WARNING:

- This hepatocyte preparation was prepared from fresh human tissue. All donor tissues have tested negative for pathogen by PCR for the following: HIV I/II, HTLV I/II, HBV, and HCV, however we recommend this material be considered a potential biohazard.
- Donors with positive serology for CMV are identified in the donor demographic sheet with a single asterisk. Donors with CMV serology unknown are identified with a double asterisk. Donors CMV negative for serology are unmarked.

SAFETY INFORMATION:

- This product is non-hazardous, according to US OSHA hazard communication/GHS 29CFR1910.1200 therefore, a SDS (Safety Data Sheet) is not required. Handle in accordance with good industrial hygiene and laboratory safety practices.

13 September 2024

Quality Assurance **Date**