Human Cryopreserved Hepatocytes
Lot number: HUM4061B
Date: October 19, 2014

TRL
TRIANGLE RESEARCH LABS

Cryo Characterization Report (CCR)

Lot Overview

Qualification	Catalog Number	Quantity
Cryopreserved human hepatocytes, Qualyst Transporter Certified	*HUCPQ3*	*3.2 x 10^6 viable cells/vial*

Donor Demographics

Sex	Race	Age	BMI	Tobacco Use	Alcohol Use	Drug Use	Serological Data	Cause of Death
Female	*Hispanic*	*44*	*30.3*	*No*	*No*	*No*	*HIV, HBV, HCV negative*	*Anoxia*

Additional donor demographic information, including relevant medical and medication history, is available upon request

Post-thaw Viability and Cell Quality Assessment

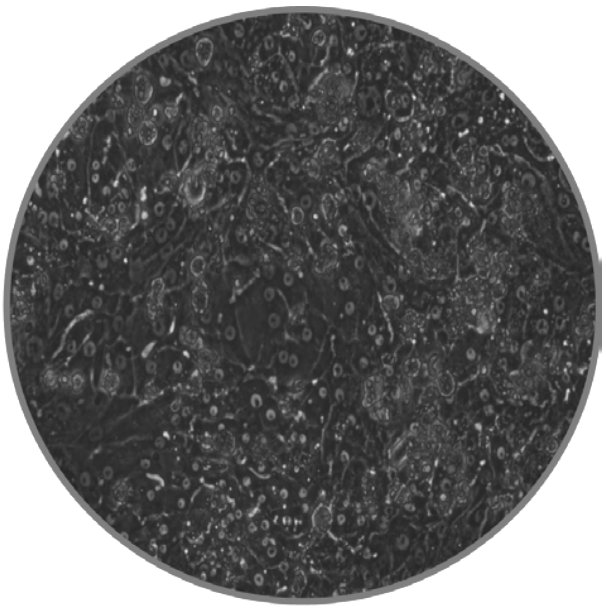
Thawing Medium Used	Centrifuge Conditions	% Viability (post-thaw)	Viable cell yield per vial
Cryopreserved Human Hepatocyte Thawing Medium	*100 x g for 8 min*	*89%*	*3.2 x 10^6*

HepatoMeter report available upon request

Monolayer Assessment

Plating Medium Used	Well Format	Culture Medium Used	Optimal Seeding Density	Initial Attachment Efficiency	Monolayer Confluency after 96hrs in culture
Human Hepatocyte Plating Medium	*24-well*	*Hepatocyte Maintenance Medium*	*0.8x10^6/mL*	*90%*	*100%*

HUM4061B, 96hrs, 10X



HUM4061B, 96hrs, 20X

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Suspension Metabolism			
Isoform	Substrate	Marker Metabolite	pmol/min/10^6 cells
General	*100 µM 7-Ethoxycoumarin*	*7-Hydroxycoumarin*	*803.7*
SULT	*100 µM 7-Hydroxycoumarin*	*7-Hydroxycoumarin Sulfate*	*14.4*
UGT	*100 µM 7-Hydroxycoumarin*	*7-Hydroxycoumarin Glucuronide*	*739.4*

Induction			
Isoforms	Control Inducer	Fold Induction Specific Activity (72 hours)	Fold Induction mRNA (48 hours)
CYP1A2	*50µM Omeprazole*	*N/A*	*8.78*
CYP2B6	*1 mM Phenobarbital*	*N/A*	*9.56*
CYP3A4	*10µM Rifampicin*	*N/A*	*26.8*

Cryopreserved human hepatocytes were thawed and plated on 24-well collagen I coated plates, overlaid with Matrigel®, then dosed in triplicate with vehicle control (0.1% DMSO) or control inducers. The fold induction was calculated by dividing the induced level by the vehicle control level.

Contact customer service to place an order or to obtain additional information on any of our lots. This may include supplementary donor demographic information, current inventory, and photomicrographs at multiple timepoints and magnifications.

To contact TRL:

Main: 800-748-8979 or 919-549-3580
Sales: 919-549-3593
Email: customerservice@triangleresearchlabs.com
Web: www.triangleresearchlabs.com

Qualyst Transporter Solutions, LLC
Better Solutions. Better Life.

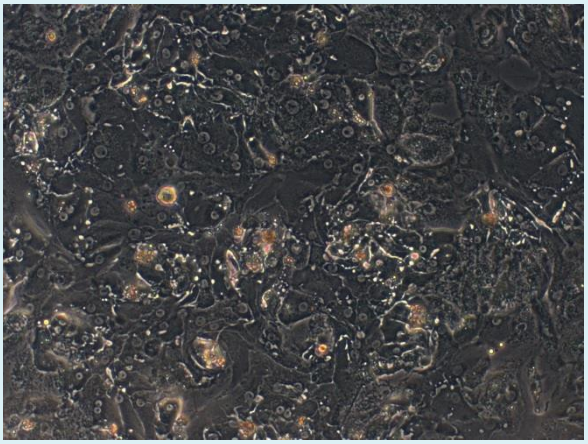
Transporter Certified™ Hepatocytes

Transporter Certification
Vendor: TRL
Lot # HUM4061B

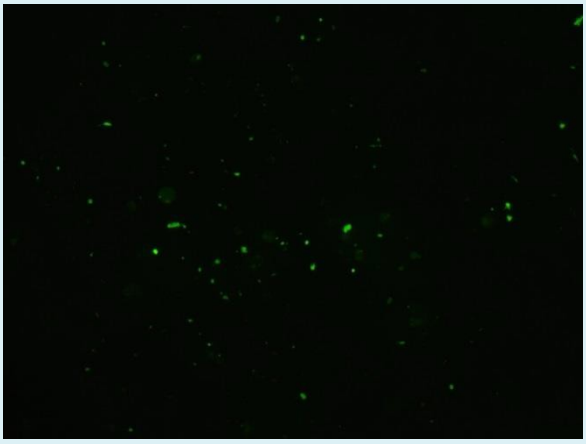
1. **Culture Characteristics**:

Characteristic	Average HUM4061B
Viability (post thaw)	84.3 %
Yield/vial (million viable cells)	3.22
Protein Content (mg/mL in 24-well)	0.115

Phase Contrast (10X)



Bile Pocket Formation (10X)



Phase-contrast image of plated sandwich-cultured hepatocytes (Day 5) using QTS proprietary media, plating, overlay and culturing protocols is shown (left). Extensive repolarization and bile pocket formation is indicated by carboxydichlorofluorescein imaging (right).

2. **Transporter Activity**: Using clinically relevant probe substrates, transport activity of uptake (accumulation) and efflux (biliary excretion) transporters is quantitated and function is compared to our historical hepatocyte database.

Probe Substrate	Hepatic Transporter	% Transported Relative to Historical Hepatocyte Database
Rosuvastatin	Uptake (OATPs)	239
Pravastatin	Uptake (OATPs)	165
Taurocholate	Uptake (NTCP, OATPs)	338
Methotrexate	Uptake (OAT1, OAT3)	**
Metformin	Uptake (OCT1)	**
Taurocholate	Efflux (BSEP)	70.2
Digoxin	Efflux (P-gp)	96.1
Metformin	Efflux (MATE1)	**
Rosuvastatin	Efflux (MRP2/BCRP)	78.7
Pravastatin	Efflux (BCRP/MRP2)	101
Methotrexate	Efflux (BCRP/MRP2)	**

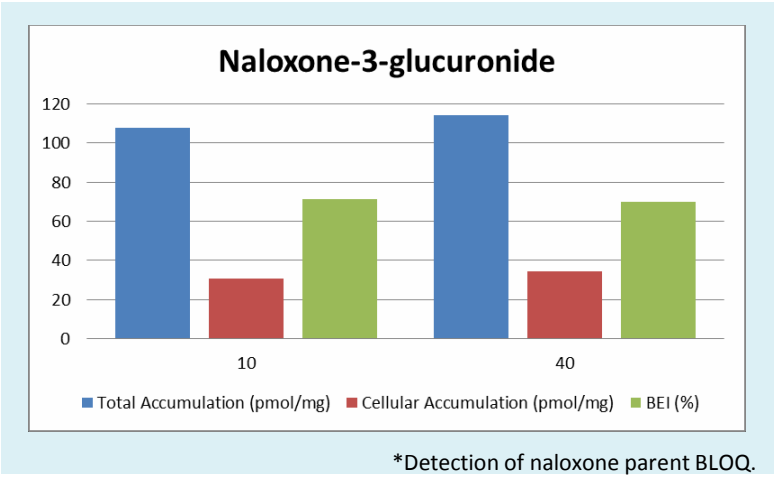
** Historical human reference data currently in progress

Percentages > 100% indicated higher transporter function compared to historical human hepatocyte database

Percentages < 100% indicated lower transporter function compared to historical human hepatocyte database

The intracellular concentration is the driving force for all of the processes that take place inside the hepatocyte. The intracellular concentration of a drug is a function of its hepatic uptake, metabolism and efflux (both basolateral and canalicular). QTS Transporter Certified™ hepatocytes are evaluated for functional uptake (accumulation) and efflux (biliary excretion) transporters using clinically relevant probe substrates covering key hepatic transporters, and compared to fresh primary hepatocytes since activity in fresh primary hepatocytes is considered the "gold standard."

3. **Conjugation Capacity and Biliary Excretion**: Metabolism of naloxone by Phase II enzymes (glucuronidation) is determined, as well as the disposition of both parent* and metabolite.



*Detection of naloxone parent BLOQ.

Measurement of metabolic capacity is key to evaluating the interaction between metabolism and transport. The metabolism of naloxone to its primary glucuronide metabolite is measured in cells and medium. The biliary excretion of naloxone glucuronide is also determined.

After 40 minutes exposure, approximately 25.8% of the naloxone in the medium had been converted to the glucuronide metabolite.

4. **Bile Acid Profile**: Human hepatocytes synthesize the primary bile acids cholic acid and chenodeoxycholic acid, and conjugate them to glycine and taurine, both in characterized ratios. These ratios are determined in QTS Transporter Certified hepatocytes that are cultured using proprietary culture conditions and medium, and ratios are compared to those reported *in vivo*.

Source	% Glycine Conjugates	% Taurine Conjugates
HUM4061B	77 %	23 %
Historical Hepatocyte Database	64 %	36 %
In Vivo	75 %	25 %

One of the major functions of the liver is to synthesize bile acids, which are secreted into the bile to aid in digestion and absorption of critical lipid-soluble nutrients. Under the correct conditions, hepatocytes can maintain this function and synthesize primary bile acids and their conjugates *in vitro*. Bile acids and their conjugates have different physicochemical properties and vary in their relative amounts *in vivo*. Therefore, *in vivo*-relevant baseline profiles of the primary bile acids and their conjugates *in vitro* are critical when using hepatocytes to evaluate a compound's potential to alter the hepatobiliary disposition of bile acids, which may lead to hepatotoxicity. Using proprietary culture medium and technology, the ratio of glycine : taurine (G:T) conjugated bile acids, are determined and compared to established *in vivo* values.

5. **Hepatic Exposure (Kp Ratio)**: The Kp ratio is the ratio of the intracellular concentration of a compound to its medium concentration following exposure for a given time. This indicates the extent of accumulation of a compound within the hepatocyte.

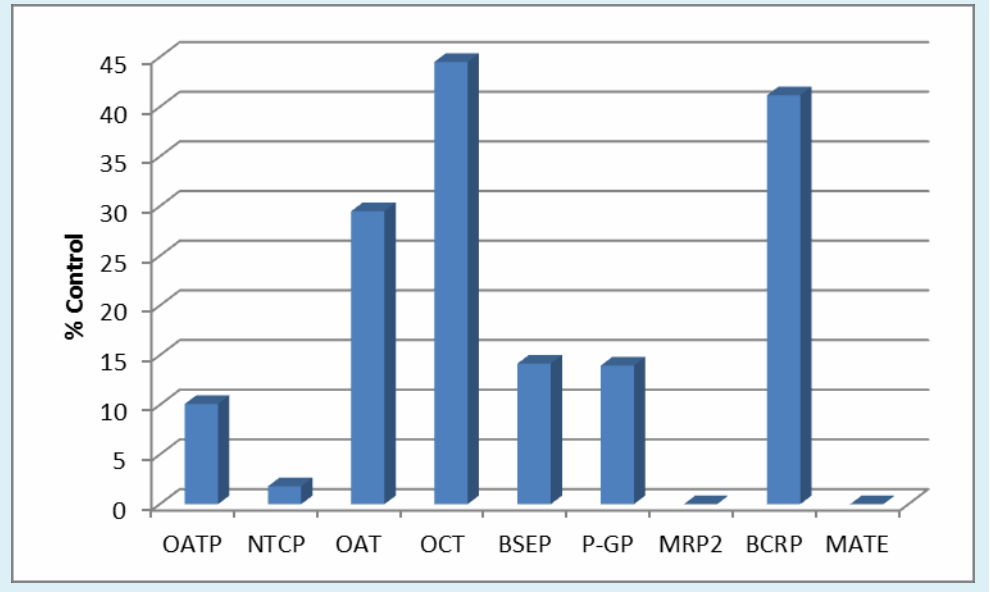
Substrate	Lot # HUM4061B Kp Ratio	Historical Hepatocyte Database Kp Ratio
Taurocholate	25.7	1.9 – 3.7
Digoxin	1.9	1.2 – 1.5
Metformin	0.7	**
Rosuvastatin	9.16	2.1 – 3.2
Pravastatin	0.9	0.3 – 0.4
Methotrexate	0.3	**

** Historical human reference data currently in progress

Compounds that are substrates for uptake transporters can accumulate significantly in the liver. The ratio of a compound's intracellular concentration to its medium concentration (Kp ratio) has been shown to correlate with the liver : blood ratio *in vivo*. A high Kp ratio suggests intracellular accumulation, and may be predictive of hepatic accumulation *in vivo*.

6. **Drug-Transporter Interactions**: Assessment of interactions between major hepatic transporters using specific, clinically-relevant probe substrates (taurocholate, digoxin, pravastatin, rosuvastatin) with and without a known broad-spectrum inhibitor (erythromycin-estolate) demonstrates specific transporter activity as well as inhibition. Results are compared to activity without inhibitor.

Effect of Erythromycin-Estolate administration on select transporter substrates in lot #HUM4061B



Transporter Certified™ hepatocytes in sandwich culture are the optimal system for evaluating the transporter interaction potential of new chemical entities. Knowledge of the potential for the function of key transporters (uptake and efflux) to be altered is critical for the evaluation of drug interactions with either other drugs or endogenous compounds.

Percent Control of Inhibition

The % control of inhibition reflects the change in activity in the presence of a broad spectrum inhibitor from control incubations without an inhibitor.

Hepatic Transporter	Transporter Function	Lot # HUM4061B (% control)	Historical Hepatocyte Database (% control)
OATP	Uptake	10.1	10.8 – 32.2
NTCP	Uptake	1.79	6.2 – 8.9
OAT	Uptake	29.5	**
OCT	Uptake	44.5	**
BSEP	Efflux (Canalicular)	14.2	75.4 – 107.2
P-gp	Efflux (Canalicular)	13.9	10.8 – 27.8
MRP2	Efflux (Canalicular)	0.0	0.0 – 33.3
BCRP	Efflux (Canalicular)	41.2	0.0 – 79.9
MATE	Efflux (Canalicular)	0.0	**

** Historical human reference data currently in progress

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