Qualyst Transporter Solutions
In Vitro Hepatic Models

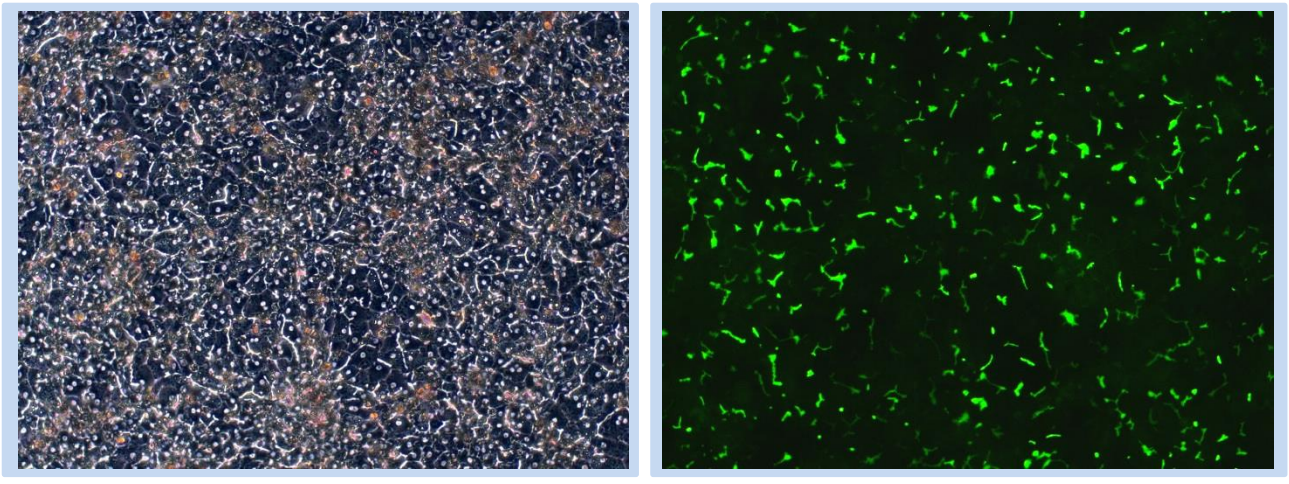
Transporter Certified™ Hepatocytes

Vendor: Lonza
Lot #: HUM4180A Male Caucasian

Culture Characteristics

Characteristic	Average
Average Yield per Vial	7.2 x 10^6 cells
Average Post-Thaw Viability	95%
Protein Content (µg/well in 24-well)	168

Cryopreserved hepatocytes were thawed following manufacturer's thawing instructions in QTS proprietary thaw medium. Hepatocytes were suspended in QTS propriety hepatocyte seeding medium (QualGro™ Seeding Medium) at a density of 0.8 million viable cells/mL onto BioCoat® 24-well cell culture plates. Eighteen to 24 hours later, cells were fed and over-laid with QualGro™ Human Hepatocyte Culture Medium supplemented with extracellular matrix (ECM), Matrigel® (0.35 mg/mL). Sandwich culture hepatocytes were maintained in QualGro™ Hepatocyte Culture Medium for five days prior to assessment of transporter function.



Phase-contrast image of plated sandwich-cultured hepatocytes (Day 5) using QTS proprietary media, plating, overlay and culturing protocols is shown (left). Hepatocyte repolarization and bile pocket formation is shown utilizing CDFDA staining (right).

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.

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."

Probe Substrate	Hepatic Transporter	% Transported Relative to QTS Historical Fresh Database
Rosuvastatin	Uptake (OATPs)	241
Pravastatin	Uptake (OATPs)	88
Taurocholate	Uptake (NTCP, OATPs)	355
Taurocholate	Efflux (BSEP)	67
Digoxin	Efflux (P-gp)	72
Rosuvastatin	Efflux (MRP2, BCRP)	79
Pravastatin	Efflux (BCRP, MRP2)	134

Transport function is scaled to fresh human hepatocyte averages based on QTS' historical database. Percentages > 100% indicated higher transporter function compared to fresh donors. Percentages < 100% indicated lower transporter function compared to fresh donors.

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. Fresh human data is provided for reference and is *not* considered acceptance criteria; values shown represent 1st and 3rd quartiles.

Transporter Substrate	Lot HUM4180A Kp Ratio	QTS Historic Fresh Database Kp Ratio
Taurocholate	26	1.74 – 7.81
Digoxin	2.78	0.566 – 6.76
Rosuvastatin	8.43	1.55 – 6.05
Pravastatin	0.759	0.241 – 0.906

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 to blood ratio in vivo. A high Kp ratio suggests intracellular accumulation, and may be predictive of hepatic accumulation in vivo.

Conjugation Capacity and Basolateral Excretion

Measurement of metabolic capacity is key to evaluating the interaction between metabolism and transport. This is evaluated through exposing cells to naloxone and quantitating its' primary metabolite in cell culture medium following incubation. Any conversion demonstrates uptake transporter function (naloxone), phase II metabolism (conversion to naloxone-3-glucuronide), and basolateral efflux transporter function (media presence of naloxone-3-glucuronide). After 40 minutes' exposure, approximately 16% of the naloxone in the medium had been converted to the glucuronide metabolite.

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
Lot HUM4180A	90 %	10 %
QTS Historical 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 to taurine (G:T) conjugated bile acids, are determined and compared to established in vivo values.

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.

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.

Effect of Inhibitor on Hepatic Transporter Function	Lot HUM4180A (% Untreated)	QTS Historical Fresh Database (% Untreated)
Uptake (OATP)	9.8%	8.8 – 34.7 %
Uptake (NTCP)	1.3%	3.1 – 8.9 %
Canalicular Efflux (BSEP)	24.9%	0.0 – 75.7 %
Canalicular Efflux (P-gp)	42.2%	6.4 – 27.8 %
Canalicular Efflux (MRP2)	0.0%	0.0 – 44.8 %
Canalicular Efflux (BCRP)	6.2%	0.0 – 79.9 %

The % untreated reflects the change in transporter function in the presence of an inhibitor with respect to control incubations (without an inhibitor), as a measure of the degree to which transporter function has been inhibited. Ranges for percent function remaining following treatment with the same broad spectrum inhibitor in the QTS Historical Fresh Database are provided for reference and is *not* considered acceptance criteria; values shown represent 1st and 3rd quartiles.

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