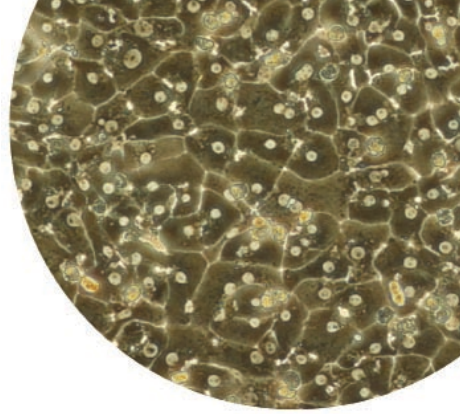
Product Characterization Sheet

Human cryopreserved hepatocytes

Lot number: Hu4175, Hu4239, Hu4244*



Donor demographics

Species	Sex	Race	Age	BMI	Smoker	Alcohol use	Drug use	Medications	Serological data	Cause of death
Human	Male	Caucasian	3	15.0	No	No	No	None listed	CMV+	Head trauma

Post-thaw viability and cell quality assessment

Thawing medium used	Optimal centrifuge conditions	% Viability (post-thaw)	Viable cell yield per vial
CHRM	100 x *g* for 10 min at room temperature	84%	6.6×10^6

Monolayer assessment

Plating medium used	Well format	Culture medium used	Optimal seeding density	Monolayer confluency at attachment	Monolayer confluency after 120 hr in culture
Williams' Medium E	24-well hand-coated plate	Williams' Medium E	0.8×10^6 cells/ml	65%	92%

Ordering Information

Product	Quantity	Cat. no.
Cryopreserved human hepatocytes	6.6×10^6 cells/1.5 ml vial	HMCPTSA

To place an order or inquire about our products and services, contact us by phone: 866 952 3559 (toll free in the USA) or +1 919 237 4679, email: hepaticproducts@invitrogen.com, or visit us on the web at www.invitrogen.com/admetox.

invitrogen™

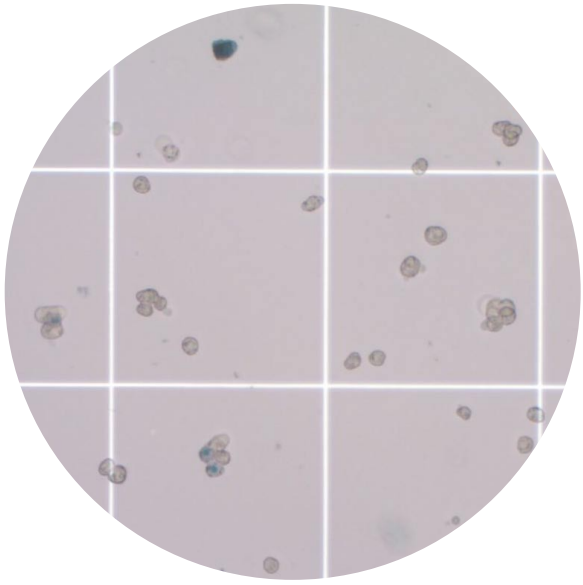
Transporter activity

	Uptake (pmol/min/mg)
Taurochloate	14.4
Digoxin	2.74
E2-17G	2.64

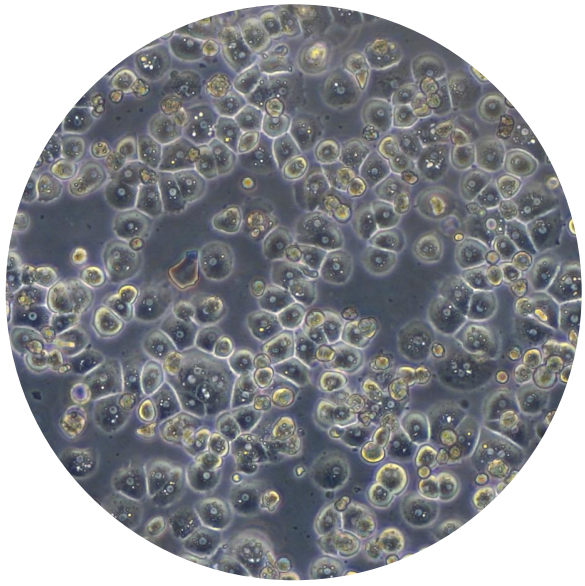
Genotyping results

Lot no.	CYP2C9	CYP2C19	CYP2D6	CYP3A5
Hu4175, Hu4239, Hu4244	WT/*3	None detected	*4/*4, WT/*9	*3/*3

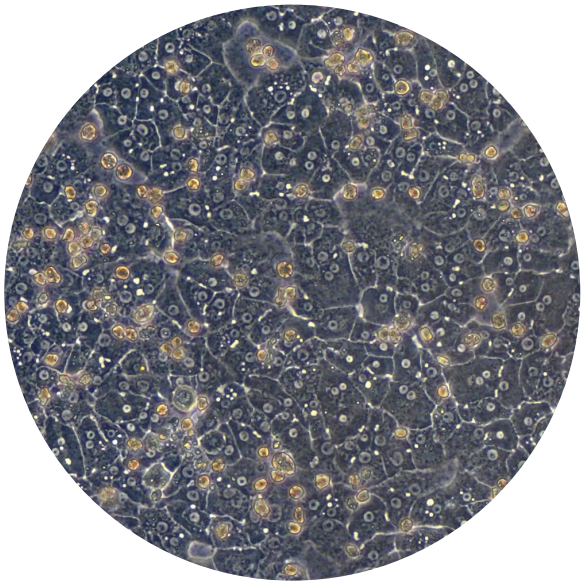
Photomicrographs of Hu4175, Hu4239, Hu4244



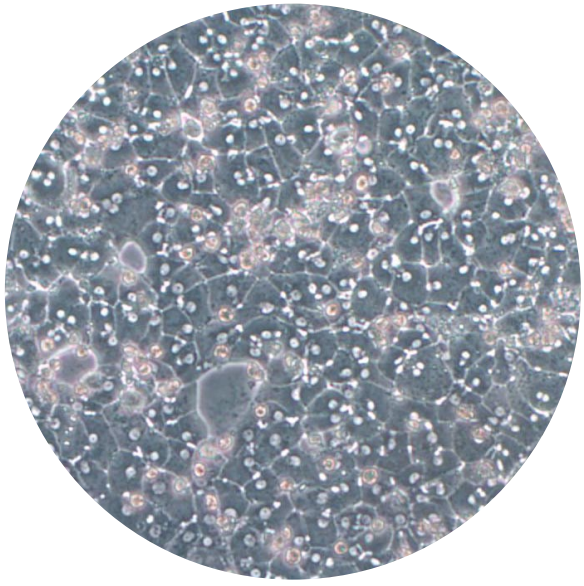
Post-thaw (10x)



5 hours after plating (24-well, 10x)



Day 3 (24-well, 10x)



Day 5 (24-well, 10x)

Transporter activity

Transporter function for these lots was assessed for uptake. Cryopreserved Human Hepatocytes were thawed in CHRM™, re-suspended in serum-containing Plating Medium and plated at 0.8 x 10^6 cells/mL in a 24-well hand-coated plate with simple collagen type I substratum. Cells were allowed to attach for 4-6 hours before an ECM gel or Geltrex™ overlay was added to the culture vessels. The plates were immediately returned to a humidified incubator at 37°C, 95% relative humidity and 5% CO_2. The medium was refreshed daily with Cell Culture Media and the condition of the sandwich cultures monitored visually using phase contrast microscopy. The transporter assays were performed on Day 5. Rates of substrate uptake were determined by the use of buffer with calcium [Plus (+) Buffer]. Hepatocyte cultures were incubated in triplicate with radiolabeled taurocholate, digoxin and estradiol 17β glucuronide (E2-17G), substrates of the uptake transporters NTCP, OATP1B3 and OATPs, respectively. Substrate concentrations and incubation times are listed in the table below. To account for non-specific binding of the radiolabeled substrate, a negative control plate absent of cells was included. Cells were lysed following incubations and samples analyzed by use of a liquid scintillation counter. Accumulation rates were determined and reported in units (pmol/min/mg).

Table 2— Incubation conditions for the transporter assay.

Substrate	Concentration (µM)	Incubation times (min)
Taurocholate	1	10
Digoxin	1	10
E2-17G	1	10

Genotyping

Genetic polymorphisms in metabolic enzymes such as CYP's can affect the way an individual responds to drug therapies. In some cases, an adjustment in dose will be necessary to elicit response, while in others, a drug may need to be replaced entirely because of a genetic polymorphism. Hepatic *in vitro* assays which employ genotyped hepatocytes can be used to study drug disposition in certain individuals with inherent SNPs. Invitrogen screens donor tissues for thirteen different SNPs within four drug-metabolizing genes. These include the following: CYP2C9*2, CYP2C9*3 , CYP2C9*6, CYP2C19*2, CYP2C19*3, CYP2C19*6, CYP2D6*3, CYP2D6*4, CYP2D6*6, CYP2D6*9, CYP3A5*3, CYP3A5*6, and CYP3A5*8. All SNPS were identified by qRT-PCR with Taqman® primer/probe sets.

References

1. FDA. (1997) Guidance for Industry—Drug metabolism/drug interaction studies in the drug development process: Studies *in vitro*, Food and Drug Administration Publication.
2. Xu, L. et al. (2000) 2,3,7,8 Tetrachlorodibenzo-p-dioxin induction of cytochrome P4501A in cultured rat and human hepatocytes. *Chem Biol Interact* 124:173–189.
3. LeCluyse, E. (2001) Pregnane X receptor: Molecular basis for species differences in CYP3A induction by xenobiotics. *Chem Biol Interact* 134:283–289.
4. LeCluyse, E.L. (2001) Human hepatocyte culture systems for the *in vitro* evaluation of cytochrome P450 expression and regulation. *Eur J Pharm Sci* 13:343–368.
5. Maurel, P. (1996) The use of adult human hepatocytes in primary culture and other *in vitro* systems to investigate drug metabolism in man. *Adv Drug Del Rev* 22:105–132.
6. Hamilton, G.A. et al. (2001) Regulation of cell morphology and cytochrome P450 expression in human hepatocytes by extracellular matrix and cell–cell interactions. *Cell Tissue Res* 306:85–99.
7. LeCluyse, E. et al. (2000) Expression and regulation of cytochrome P450 enzymes in primary cultures of human hepatocytes. *J Biochem Mol Toxicol* 14:177–188.
8. Wang, H. and LeCluyse, E. (2003) Role of orphan nuclear receptors in the regulation of drug metabolizing enzymes. *Clin Pharmacokinet* 42:1331–1357.
9. LeCluyse, E.L. et al. (2005) Isolation and culture of primary human hepatocytes. *Methods Mol Biol* 290:207–229.
10. Bjornsson, T. et al. (2003) The conduct of *in vitro* and *in vivo* drug–drug interaction studies: a Pharmaceutical Research and Manufacturers of America (PhRMA) perspective. *Drug Metab Dispos* 31:815–832.

* Preliminary data, intended for marketing purposes and lot identification only. Final data will be released through ADME/Tox Quality Systems.
For quality certificates, please contact your local sales representative or call 1 866-952-3559.