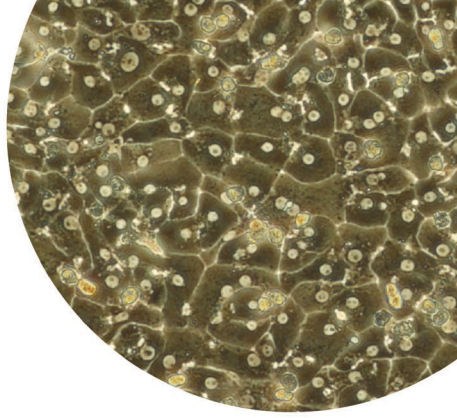
Product Characterization Sheet

Human cryopreserved hepatocytes
Lot number: Hu8055*



Donor demographics

Species	Sex	Race	Age	BMI	Smoker	Alcohol use	Drug use	Medications	Serological data	Cause of death
Human	Female	Caucasian	40 years	36.4	Yes	Yes	No	*	All negative	CVA

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	92%	5.8×10^6

Monolayer assessment

Plating medium used	Well format	Culture medium used	Optimal seeding density	Initial attachment efficiency	Monolayer confluency after 72 hr in culture
Williams' Medium E	24-well hand-coated plate	Williams' Medium E	0.9×10^6 cells/ml	80%	75%

Ordering Information

Product	Quantity	Cat. no.
Cryopreserved human hepatocytes	5.8×10^6 cells/1.5 ml vial	HMCPMS

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.

*Topamax, Cymbalta, synthroid, Wellbutrin, Pentasa, adderall, Valtrex, Ketek, Prednisone

invitrogen™

Metabolic profile (pmol/min/10^6 cells)

CYP1A2	CYP2B6	CYP2C8	CYP2C9	CYP2C19	CYP2D6	CYP2E1	CYP3A Testosterone	CYP3A Midazolam	FMO
20.1	244	NA	103	87.1	15.6	NA	1,039	512	NA

7-Ethoxycoumarin-O-deethylase (ECOD)	7-Hydroxycoumarin Glucoronidation (Phase II)	7-Hydroxycoumarin Sulfation (Phase II)
28.5	699	17.2

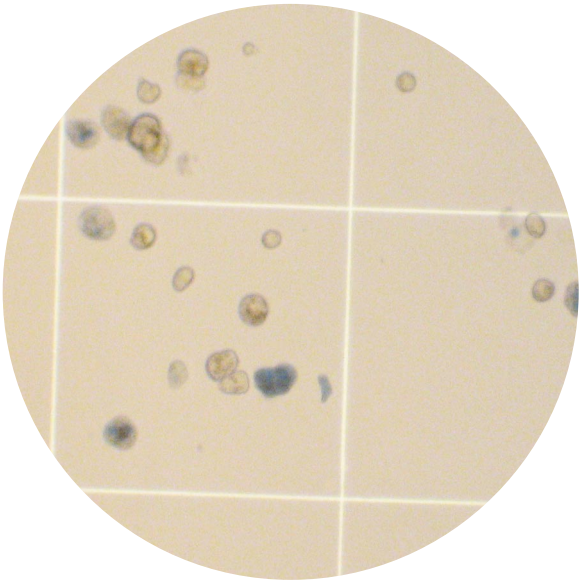
Plated metabolism (Intrinsic clearance) (µL/min/10^6 cells)

Midazolam	Tolbutamide	Dextromethorphan
5.91	0.29	1.29

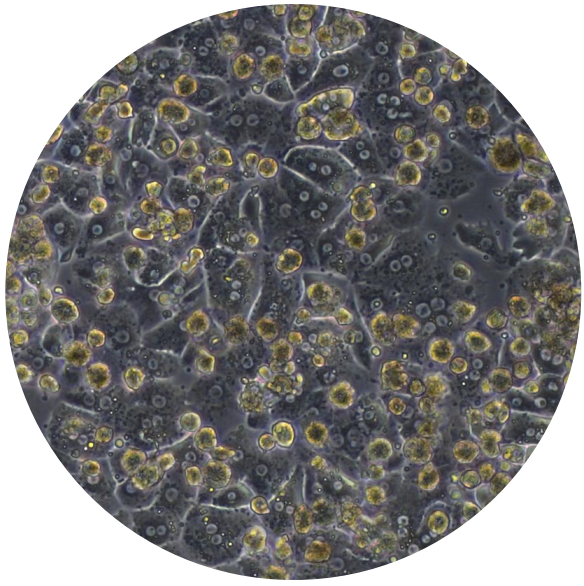
Genotyping results

Lot no.	CYP2C9	CYP2C19	CYP2D6	CYP3A5
Hu8055	WT/WT	WT/WT	WT/*4	*3/*3

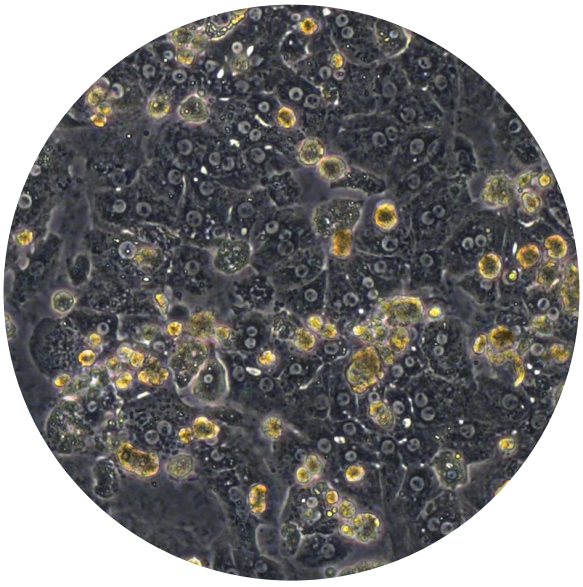
Photomicrographs of Hu8055



Post-thaw (10x)



Day 2 (24-well: 10x)



Day 3 (24-well: 10x)

Metabolic assay conditions

The commonly tested Phase 1 (P450) and Phase II (UGT, SULT) drug metabolizing enzymes were analyzed for metabolic activity in lot Hu8055. Cells were carefully thawed in CHRM and resuspended to 1.0 x 10^6 cells/ml in Incubation Medium (serum-free). 0.25 mL or 0.5 mL (7-EC, 7-HC) of cell suspension was aliquoted into a 24-well or a 12-well non-coated plate, respectively, containing the appropriate substrate and placed in a humidified incubator at 37°C, 95% relative humidity, and 5% CO_2 on an orbital shaker. All incubations were performed in triplicate. At the appropriate time point for each substrate, a sample was collected from each well and stored frozen at –70°C until processed by LC/MS/MS or HPLC analysis. Metabolite formation was measured by standard biochemical assays using GLP-validated LC/MS/MS assays. At least six calibration standards and 12 quality control samples (at three different concentrations) were used to evaluate the quality of analytical runs.

Table 1—Substrate probes for the assessment of human CYP450 activity.

Enzyme	Substrate	Concentration (μM)	Incubation time	Marker metabolite
CYP1A2	Phenacetin	100	15 min	Acetaminophen
CYP2B6	Bupropion	500	15 min	Hydroxybupropion
CYP2C8	Paclitaxel	20	30 or 45 min	6α-Hydroxypaclitaxel
CYP2C9	Diclofenac	25	15 min	4′-Hydroxydiclofenac
CYP2C19	(S)-Mephenytoin	250	30 min	4′Hydroxymephenytoin
CYP2D6	Dextromethorphan	15	15 min	Dextrorphan
CYP2E1	Chlorzoxazone	250	15 min	6-Hydroxychlorzoxazone
CYP3A	Testosterone	200	15 min	6β-Hydroxytestosterone
CYP3A	Midazolam	10	10 min	1-Hydroxymidazolam
Phase I	7-Ethoxycoumarin	100	30 min	(ECOD) 7-Hydroxycoumarin (7-HC)
Phase II	7-Hydroxycoumarin	100	30 min	7-Hydroxycoumarin Glucuronide (7-HCG) and 7-Hydroxycoumarin Sulfate (7-HCS)
FMO	Benzydamine	250	30 min	Benzydamine-N-Oxide

Metabolic assay conditions

Cryopreserved Human Hepatocytes were seeded in 48-well coated plates at 0.8 x 10^6 cells/mL (unless otherwise noted) and allowed to attach prior to metabolic incubations. Prototypical cytochrome P450 substrates midazolam, tolbutamide, and dextromethorphan were used to assess the enzymatic function of CYP3A4/5, CYP2C9 and CYP2D6 respectively. The concentrations and incubation times are included in the chart below. Incubations were conducted in duplicate in serum-free Williams Medium E culture medium and reactions allowed to proceed in a humidified incubator at 37°C, 95% relative humidity, and 5% CO_2 on an orbital shaker. Reactions were stopped with the addition of ice-cold acetonitrile. Well contents were stored at -70°C prior to analysis. The disappearance of parent was monitored by LC-MS/MS analysis and intrinsic clearance (CL_{int}) values determined by linear regression.

Table 2—Incubation conditions for CL_{int} in plated cryopreserved human hepatocytes.

Substrate	Concentration (µM)	Incubation Time (h)
Midazolam	0.50	0,1,2,4,6,8
Tolbutamide	1.00	0,4,6,8,18,24
Dextromethorphan	1.00	0,1,2,4,6,8

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.