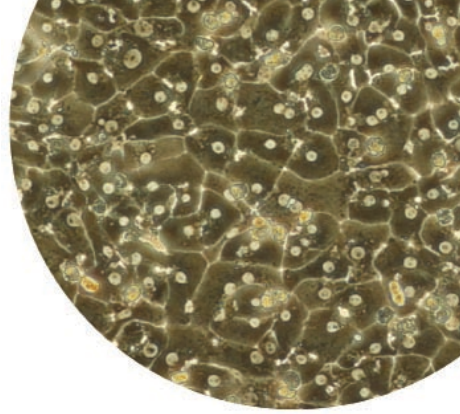
Product Characterization Sheet

Human cryopreserved hepatocytes

Lot number: Hu4242*



Donor demographics

Species	Sex	Race	Age	BMI	Smoker	Alcohol use	Drug use	Medications	Serological data	Cause of death
Human	Male	Caucasian	21	28.0	Yes	Yes	No	Lamictol	All negative	Anoxia

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	86%	5.7×10^6

Monolayer assessment

Plating medium used	Well format	Culture medium used	Optimal seeding density	Monolayer confluency at attachment	Monolayer confluency after 48 hr in culture
Williams' Medium E	24-well hand-coated plate	Williams' Medium E	0.8×10^6 cells/ml	60%	70%

Ordering Information

Product	Quantity	Cat. no.
Cryopreserved human hepatocytes	5.7×10^6 cells/1.5 ml vial	HMCPMS

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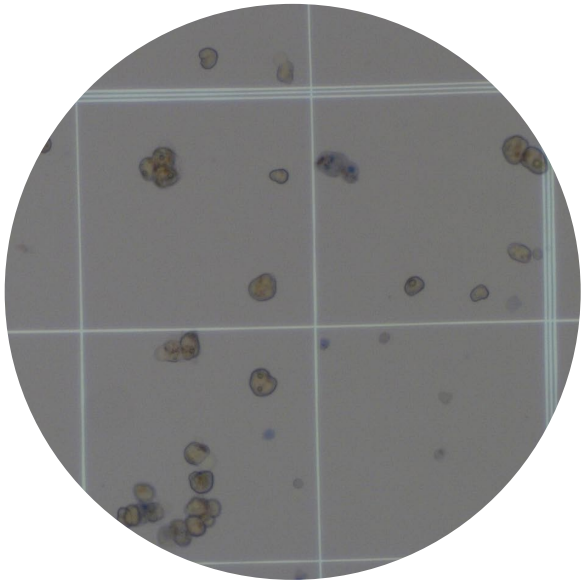
Plated metabolism (Intrinsic clearance) - µL/min/10^6 cells

Midazolam	Tolbutamide	Dextromethorphan
8.88	0.335	5.20

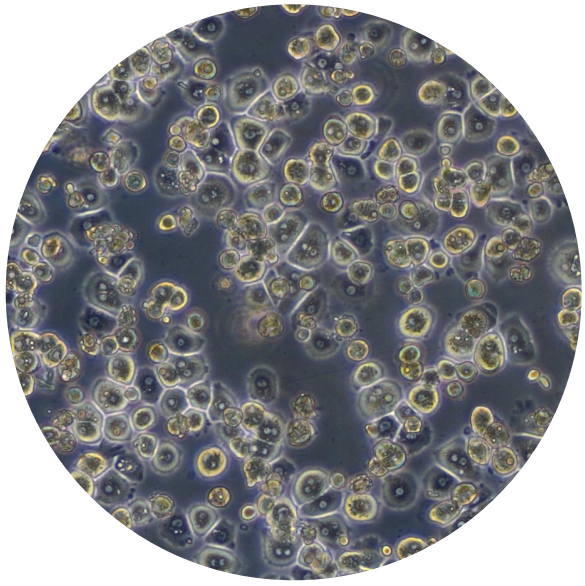
Genotyping results

Lot no.	CYP2C9	CYP2C19	CYP2D6	CYP3A5
Hu4242	TBD	TBD	TBD	TBD

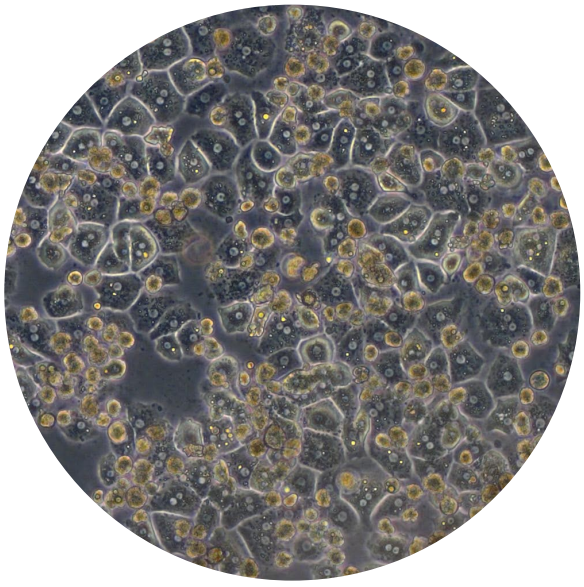
Photomicrographs of Hu4242



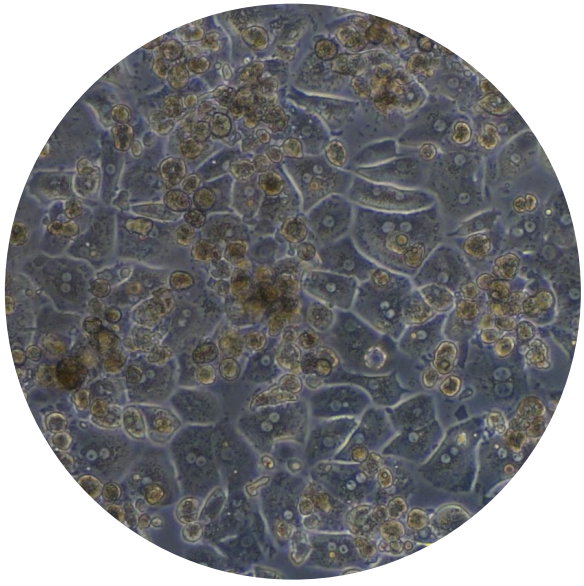
Post-thaw (10x)



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



Day 2 (24-well, 10x)



Day 2 (48-well, 10x)

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

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* Preliminary data, intended for marketing purposes and lot identification only. Final data will be released through ADME/Tox Quality Systems.
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