Product Characterization Sheet

Human cryopreserved hepatocytes
Lot number: Hu8080
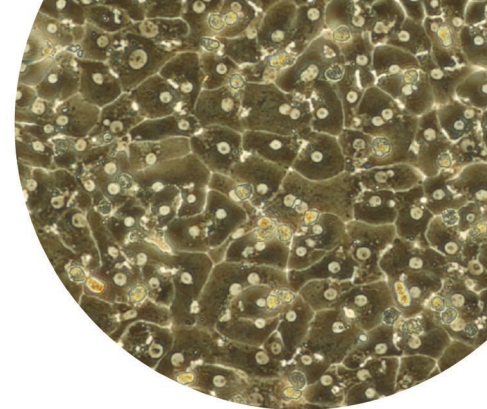
Application recommendations

Enzyme induction	Recommended

Inventory information

Current available inventory	575

Donor demographics

Species	Sex	Race	Age	BMI	Smoker	Alcohol use	Drug use	Medications	Serological data	Cause of death
Human	Male	Caucasian	19 years	25.0	Yes	Yes	Yes *	None listed	CMV+	GSW to head

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	95%	5.0×10^6

Monolayer assessment

Plating medium used	Well format	Culture medium used	Optimal seeding density	Initial attachment efficiency	Monolayer confluency after 120 hr in culture
Williams' Medium E	24-well hand-coated plate	Williams' Medium E	0.80×10^6 cells/ml	65%	90%

Ordering Information

Product	Quantity	Cat. no.
Cryopreserved human hepatocytes	5.0×10^6 cells/1.5 ml vial	HMCPIS

*Marijuana and pain killers

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.

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Fold induction (specific activity)

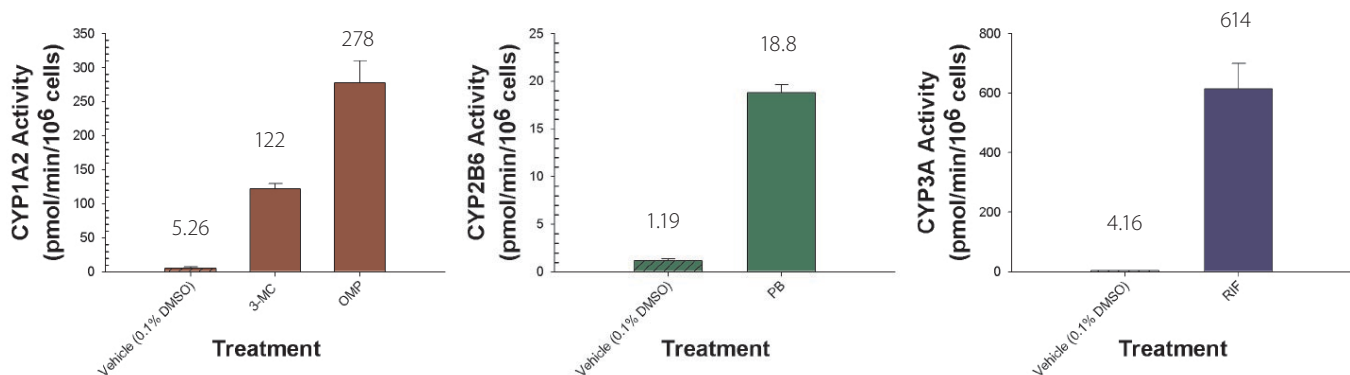| CYP1A2:	3-MC (2 µM)	OMP (50µM)	CYP2B6:	PB (1000 µM)	CYP3A:	RIF (10 µM)
	23.2	52.9		15.8		148

Figure 1 - Vehicle control and induced CYP activities of lot Hu8080. Cryopreserved Human Hepatocytes (Hu8080) were cultured in 24-well collagen-coated plates and dosed in triplicate with vehicle (0.1% DMSO), 3-methylchloranthrene (3-MC), omeprazole (OMP), phenobarbital (PB) and rifampin (RIF) for 72 hours. Monolayers were washed and incubated with substrates phenacetin, bupropion and testosterone to determine CYP1A2, CYP2B6 and CYP3A specific activities, respectively. An average of three independent samples ± standard deviation are depicted in the bar graphs. Fold-induction of specific activity is expressed as the ratio of induced activity to vehicle activity.
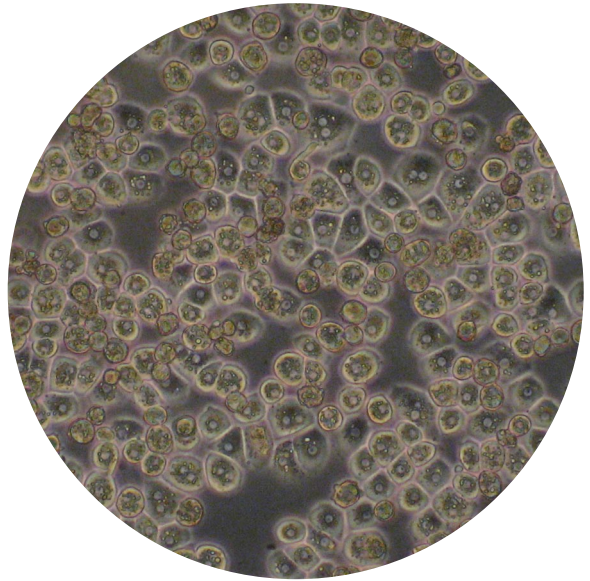
Fold induction (mRNA)

CYP1A2:	3-MC (2 µM)	OMP (50µM)	CYP2B6:	PB (1000 µM)	CYP3A4:	RIF (10 µM)
	125	130		12.6		755

Genotyping results

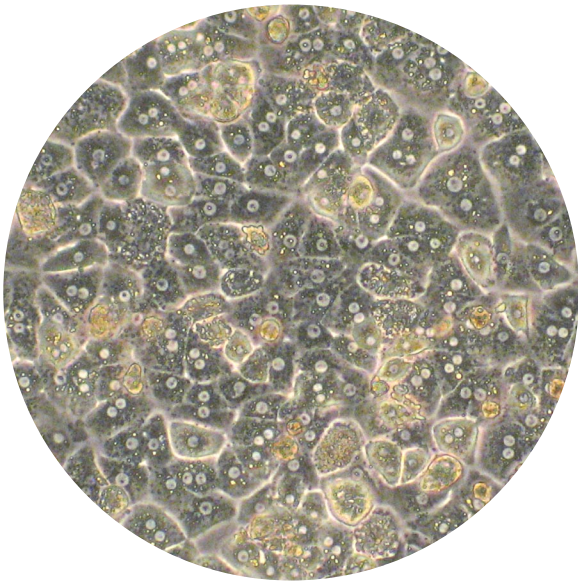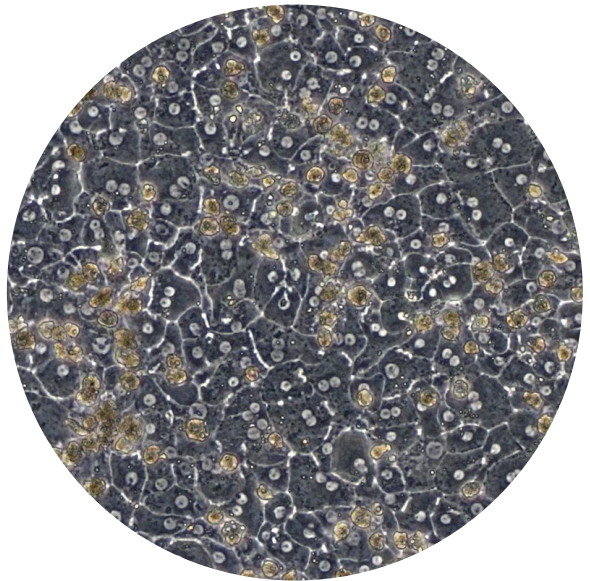| Lot no.	CYP2C9	CYP2C19	CYP2D6	CYP3A5
Hu8080	WT/*3	WT/*2	*4/*4	*3/*3

Photomicrographs of Hu8080
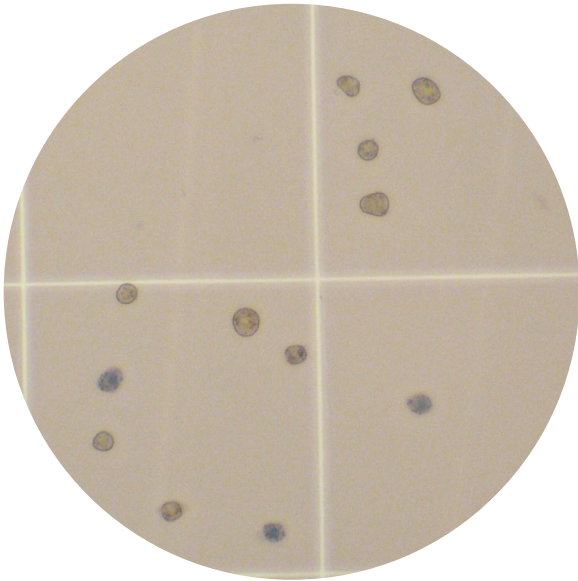
Hu8080—Post-thaw (10x)

Hu8080—5 hours after plating (24-well: 10x)

Hu8080—Day 2 (24-well: 10x)

Hu8080—Day 5 (24-well: 10x)

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Enzyme induction assay

A focused strategy based on the underlying mechanisms of drug-induced gene regulation was used to assess whether **Hu8080** is an inducible lot of hepatocytes suitable to be used in experiments monitoring *in vitro* enzyme induction. Our strategy was to monitor changes in the expression and activity of single, sensitive target genes for each of the major drug-induced nuclear receptors. We incorporated a select panel of target genes, CYP1A2, CYP2B6, and CYP3A, for this assessment. Using this strategy we evaluated the potential of known enzyme inducers represented by 3-methylcholanthrene (3-MC; a prototypical CYP1A enzyme inducer), omeprazole (OMP; a prototypical CYP1A enzyme inducer), phenobarbital (PB; a prototypical CYP2B inducer), and rifampicin (RIF; a prototypical CYP3A enzyme inducer) to verify that **Hu8080** is an inducible lot of hepatocytes.

Cells were first thawed using Cryopreserved Hepatocyte Recovery Medium (CHRM) and plated using Williams' Medium E (WEM) (serum-containing) at the predetermined optimal density of 0.80 x 10^6 cells/ml in a 24-well plate hand-coated with simple type I collagen. Cells were allowed to attach for 4-6 hrs before overlay with Geltrex™ in serum-free WEM containing ITS+. Plates were maintained in an atmosphere of 37°C, 95% relative humidity, and 5% CO_2 for approximately 24 hrs prior to treatment with the clinically relevant inducers. The medium was replaced daily with fresh supplemented medium or medium containing the inducers, as appropriate. Cell morphology and monolayer integrity were checked prior to initiating the experiment with the substrates to ensure that the monolayers were of acceptable quality for the study.

Hepatocyte cultures were then treated for 2 consecutive days (RNA isolations) and 3 consecutive days (*in situ* incubations) at one concentration per inducer in triplicate. After completion of the treatment period, the medium was aspirated from the plates, and the cell monolayers were rinsed with Hank's Balanced Salts Solution (HBSS). HBSS containing the appropriate CYP450 marker substrates for CYP1A2, CYP2B6, and CYP3A was added directly to the monolayers (Table 1). Plates were incubated at approximately 37°C in a humidified chamber while mixing on an orbital shaker. At the end of the incubation periods, samples were collected and stored frozen at –70°C until they were processed for LC-MS/MS analysis. Metabolite formation was measured by standard biochemical assays using GLP-validated LC-MS/MS assays. At least 6 calibration standards and 12 quality control samples (at 3 different concentrations) were used to evaluate the quality of the analytical runs. The extent of induction was evaluated by comparing the normalized enzyme activities of the inducer-treated cells to those of the vehicle control (0.1% DMSO only) and calculating fold induction.

Additionally, relative mRNA was analyzed using TaqMan® methodology. At the end of the 48 hr treatment period, dosed cells to be used for RNA isolation were washed with HBSS and subsequently lysed according to the recommended procedures for the ABI PRISM® 6100 Nucleic Acid PrepStation (Applied Biosystems). Isolated RNA was analyzed on a NanoDrop® spectrophotometer (Thermo Scientific) to evaluate RNA purity and

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

Enzyme	Substrate	Concentration	Incubation time	Marker metabolite
CYP1A2	Phenacetin	100 µM	15 min	Acetaminophen
CYP2B6	Bupropion	500 µM	20 min	Hydroxybupropion
CYP3A	Testosterone	200 µM	14 min	6β-Hydroxytestosterone

estimate the concentration of isolated total RNA. For qRT-PCR, a two-step process was used: reverse transcription (RT) was performed on isolated RNA with the High Capacity cDNA Archive Kit (Applied Biosystems); quantitative PCR analysis was performed on RT reactions using gene-specific primer/probe sets for CYP1A2, CYP2B6, and CYP3A4 target cDNA as well as an endogenous control. Samples were analyzed on a Applied Biosystems 7500 Real-Time PCR System. Relative-fold mRNA content will be determined based on threshold cycles (C_t) of the target gene, compared to an endogenous control for each reaction and normalized to vehicle control.

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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