ADME/TOX

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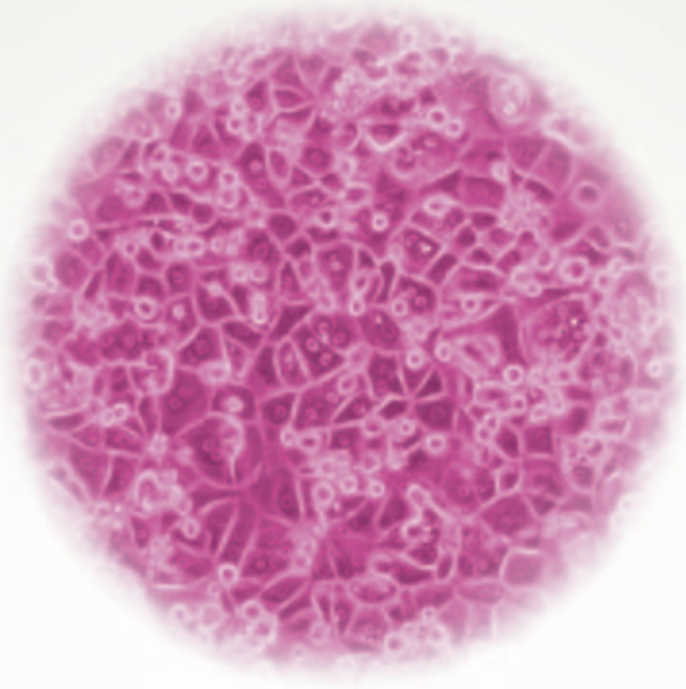

introducing **forever**

Infinitely reproducible results with cryopreserved HepaRG™ cells

life technologies™

When you need reproducible drug metabolism or toxicity data, HepaRG™ cells are the *in vitro* tool that provides consistent results in a metabolically complete and scalable system. HepaRG™ cells are terminally differentiated hepatic cells derived from a human liver progenitor cell line. As a result, they retain many critical primary human hepatocyte characteristics, without the limitations of primary cells such as donor variability, short life span, and limited availability. HepaRG™ cells are provided in a convenient cryopreserved format, and are appropriate for many applications, including:

Induction screening

A unique *in vitro* system for induction screening

P450 enzymes can be induced as a result of drug exposure, which may cause increased formation of toxic metabolites and/or decreased systemic levels of a coadministered drug, potentially resulting in drug toxicity or decreased drug efficacy. The use of primary human hepatocytes in screening applications is limited by tissue availability, donor variability, cost, and a relatively short culture lifespan. The use of HepaRG™ cells solves these limitations without sacrificing critical hepatocyte traits such as drug-metabolizing enzyme expression, functional transport proteins, and expression of key nuclear receptor pathways. HepaRG™ cells respond to prototypical P450 inducers such as omeprazole (OMP), phenobarbital (PB), and rifampicin (RIF), demonstrating the utility of this cell system in the evaluation of *in vitro* enzyme induction (Figure 1).

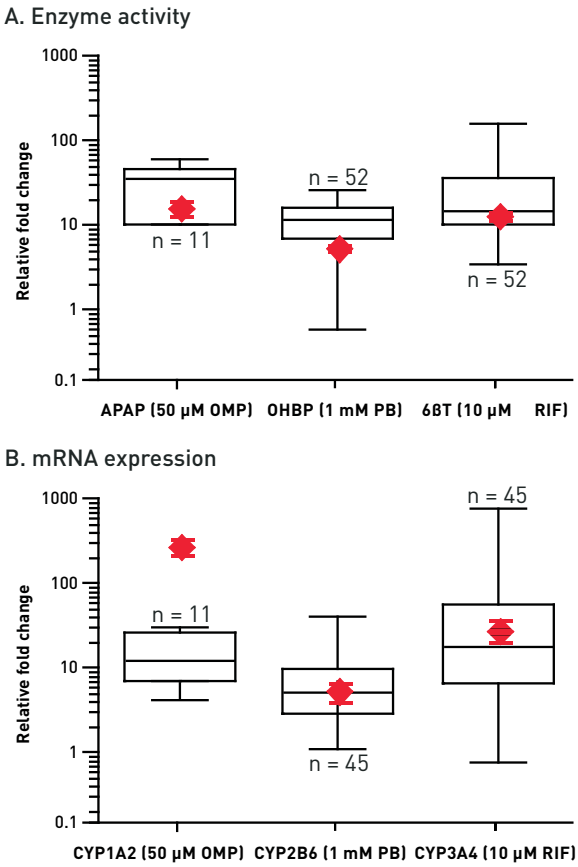

Figure 1. Induction of CYP1A2, CYP2B6, and CYP3A4 enzyme activity (A) and mRNA expression (B) in HepaRG™ cells or primary human hepatocytes (PHH) treated with omeprazole (OMP), phenobarbital (PB), or rifampicin (RIF) for 72 hr in culture. Box and whisker plots (box = 25th to 75th percentile, line within box = median, whiskers = extreme values observed) were generated using data from multiple PHH preparations, illustrating the large donor-to-donor variability observed in PHH. HepaRG™ data (activity and mRNA) for CYP1A2, CYP2B6, and CYP3A4 are denoted as red diamonds and were generated from three separate vials.

Inhibition analysis using HepaRG™ cells

Inhibition of a specific drug-metabolizing enzyme can decrease the metabolic clearance of a coadministered drug, leading to elevated blood concentrations, which may lead to adverse effects. Similar IC_{50} values were generated in both HepaRG™ cells and primary hepatocytes for certain prototypical P450 inhibitors, indicating that HepaRG™ cells are a suitable cell system to evaluate a compound's inhibition potential (Table 1).

Table 1. IC_{50} of HepaRG™ cells and PHH (mean of 2) treated for 24 hr with cocktail of substrates and inhibitors [1].

CYP	Inhibitor	HepaRG™ IC_{50}	PHH IC_{50}
1A1/2	Furafylline	<0.1	<0.1
2A6	Trancylpromine	<0.1	<0.1
2B6	Ticlopidine	<0.1	<0.1
2C8	Montelukast	2.6	4.2
2C9	Sulfaphenazole	<0.1	<0.1
2C19	Fluconazole	42.5	52.5
2D6	Quinidine	<0.1	<0.1
3A4	Ketoconazole	<0.1	<0.1

Metabolism

Assess metabolic stability

Estimates of *in vivo* metabolic drug clearance can be determined from *in vitro* metabolism kinetic data. Metabolic stability studies are typically performed to estimate a drug candidate's metabolic half-life and intrinsic clearance rates, which are major determinants of *in vivo* drug efficacy. Compounds with short half-lives may require multiple doses to maintain effective plasma levels, whereas compounds with slower elimination kinetics require fewer doses. Unlike other cell lines (e.g., HepG2 and Fa2N-4), HepaRG™ cells have expression levels of key metabolic enzymes and nuclear receptors consistent with levels observed in PHH, and therefore are more suitable for assessing the metabolic stability of candidate compounds (Figure 2).

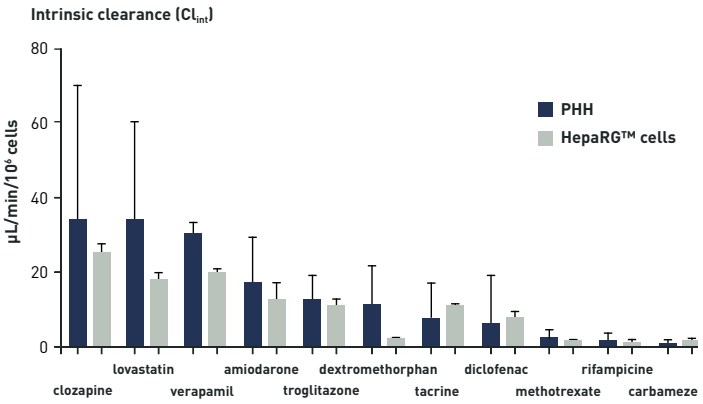

Figure 2. Intrinsic clearance of the reference drugs aminodarone, carbamezepine, clozapine, diclofenac, dextromethorphan, lovastatin, methotrexate, rifampicine, tacrine, troglitazone, and verapamil in cultures of primary human hepatocytes (PHH, n=6) and HepaRG™ cells (n = 2). Results are shown as means + SD [2].

Toxicity

Investigate acute and chronic toxicity

The liver plays a central role in metabolizing and eliminating xenobiotics and as a result is susceptible to injury from drug toxicity. Liver toxicity has led to withdrawal or severe use limitations of marketed drugs and is a major problem in drug development. HepaRG™ cells are a metabolically competent system and tolerant of long culture periods (i.e., ⩾22 days). In addition, they are well-suited for *in vitro* determinations of acute and chronic toxicity resulting from intrinsic and/or metabolism-based mechanisms (Figures 3 and 4).

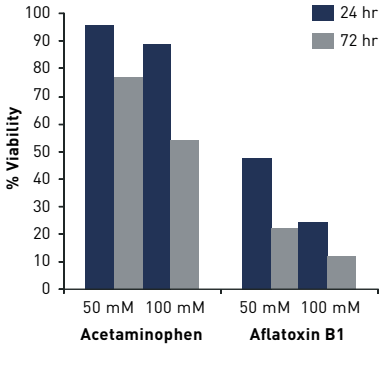

Figure 3. HepaRG™ cell viability after 24 hr and 72 hr treatment with metabolism-dependent toxicants [3].

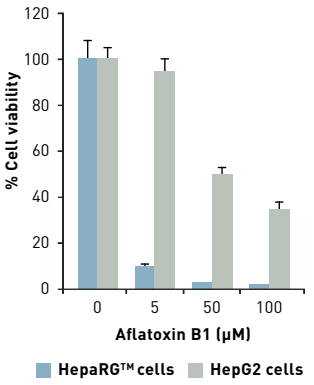

Figure 4. Comparative cytotoxicity of aflatoxin B1 in HepG2 and HepaRG™ cells after a 3-day treatment. Cell viability was estimated using a standard MTT test. The values were normalized to untreated cells and expressed as means ± SD (n = 3 cultures) [4].

Transporters

Assess potential transporter-mediated drug interactions

Transporters often work together with drug-metabolizing enzymes in drug absorption and elimination, resulting in altered drug efficacy or adverse drug effects. HepaRG™ cells have superior expression levels of key uptake and efflux transporters compared to other cell lines and expression levels closely resembling those of human hepatocytes (Figures 5 and 6).

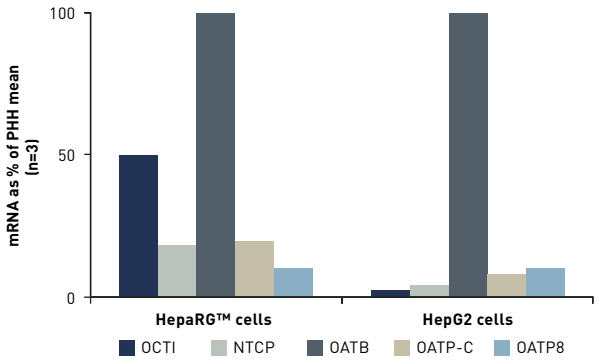

Figure 5. Uptake transporter gene expression, HepaRG™ and HepG2 cells vs. PHH [5].

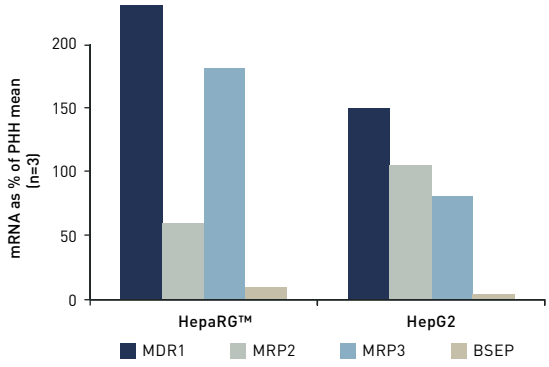

Figure 6. Efflux transporter gene expression, HepaRG™ and HepG2 cells vs. PHH [5].

Ordering information

Product	Cat. No.
HepaRG™ Cells, Cryopreserved	HPRGC1
Supplements	
HepaRG™ Maintenance/Metabolism Medium Supplement	HPRG620
HepaRG™ Tox Medium Supplement	HPRG630
HepaRG™ Induction Medium Supplement	HPRG640
HepaRG™ Serum-free Induction Medium Supplement	HPRG650
HepaRG™ Thaw, Plate, and General Purpose Medium Supplement	HPRG670
HepaRG™ Maintenance/Metabolism Medium Supplement (5x)	HPRG720
HepaRG™ Tox Medium Supplement (5x)	HPRG730
HepaRG™ Induction Medium Supplement (5x)	HPRG740
HepaRG™ Serum-free Induction Medium Supplement (5x)	HPRG750
HepaRG™ Thaw, Plate, and General Purpose Medium Supplement (5x)	HPRG770
Media and plates	
Williams' Medium E (1X) without Phenol Red, 500 mL	A1217601
Collagen I coated plate, 24-well	A1142802
Collagen I coated plate, 96-well	A1142803
GlutaMAX™-I Supplement	35050061

Contact us

To inquire about HepaRG™ cells or learn more about our products, please contact us by email at hepaticproducts@invitrogen.com
We can also be reached by phone: 866 952 3559 (toll-free in North America), +44 (0) 141 814 5900 (Europe), +1 919 237 4679 (toll number), or go to www.invitrogen.com/heparg

References

1. Aninat C et al. (2008) *Crit Care Med* 36:848–854.
2. Lübberstedt M et al. (2011) *J Pharmacol Toxicol Methods* 63:59–68.
3. Aninat C et al. (2006) *Drug Metab Dispos* 34:75–83.
4. Guillouzo A et al. (2007) *Chem Biol Interact* 168:66–73.
5. Le Vee M et al. (2006) *Eur J Pharm Sci* 28:109–117.