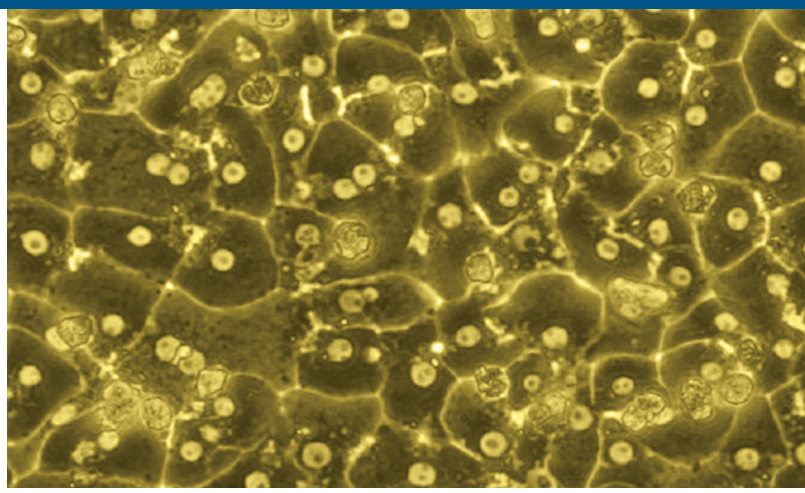
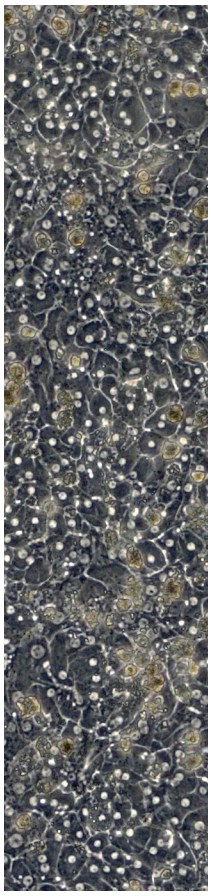
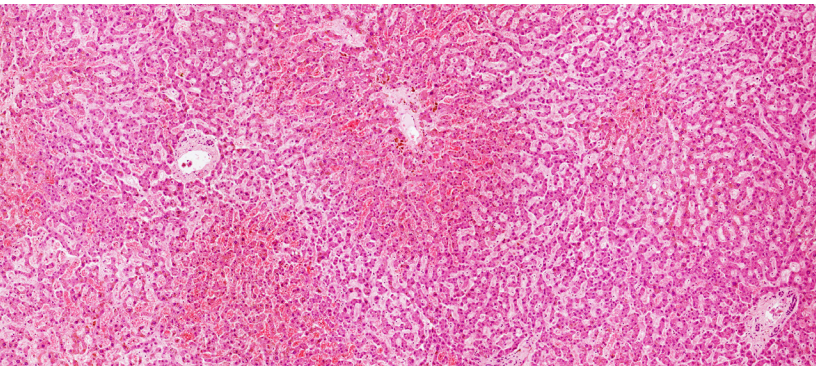
gibco



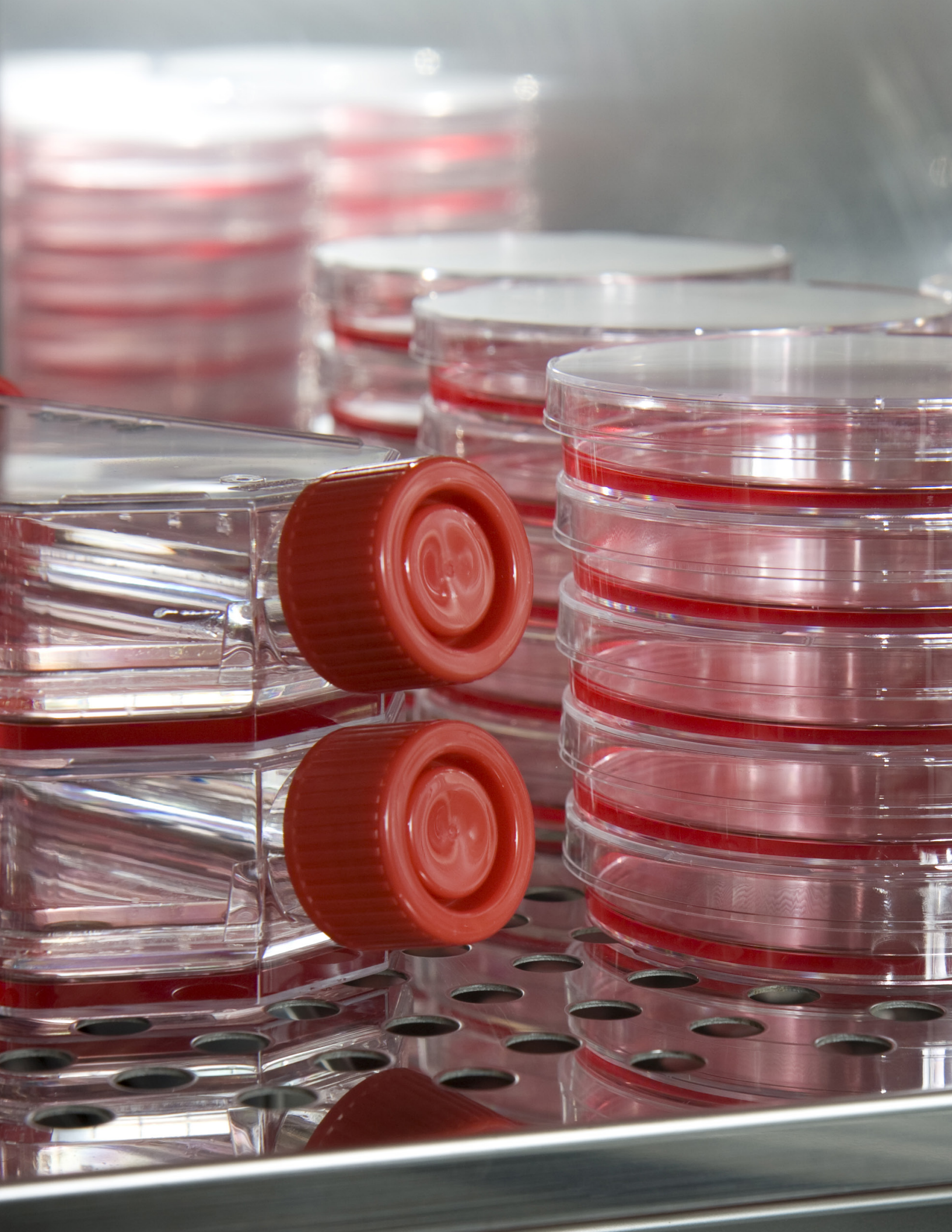




ADME/Tox sourcebook

Products to enable research and discovery

ThermoFisher SCIENTIFIC



ADME/Tox products and services

At Thermo Fisher Scientific, we strive to offer industry-leading ADME/Tox products that provide physiologically relevant results, so you can make the right decisions relating to your compounds. We are a team of hepatic scientists helping fellow scientists—supporting IND and NDA submissions with our products and services, and participating in the advancement of research in enzyme induction, CYP inhibition, hepatic transport, and other metabolism-related fields. Our goal is to provide the right tools and technical support for your needs.

ADME/Tox products for drug metabolism and safety

Physiologically relevant *in vitro* systems, such as primary hepatocytes and liver subcellular fractions, can be used to address a wide array of research questions related to *in vivo* applications, including those related to xenobiotic metabolism, drug–drug interactions, and cytotoxicity (Table 1). We offer products for:

- CYP450 inhibition and enzyme induction
- Metabolic profiling and stability
- Transporter applications

Complete selection of hepatic cell products

Our comprehensive offering includes hepatocytes and subcellular fractions isolated from human, rat, mouse, dog, and nonhuman primate. Our products are prequalified for particular research applications, and most are supplied as pooled or single-donor, often in large lot sizes to facilitate multiple and multi-site experiments. These products include:

- Cryopreserved primary hepatocytes, Kupffer cells, and stellate cells
- Gibco™ HepaRG™ cells
- Cell culture media

Gibco™ quality—every step of the way

As hepatic scientists, we deeply understand the importance of a high-quality, reliable supply chain and stringent characterization methods. We offer:

- A robust, extensive tissue procurement network
- Carefully honed isolation techniques
- Rigorous quality control standards
- Ongoing research and development
- Hepatic product specialists to provide technical support

Table 1. ADME/Tox products from Thermo Fisher Scientific and their applications.

Application	Purpose	FDA guidance*	Plateable hepatocytes
Drug development research (ADME, DMPK, toxicology)			
Enzyme induction and inhibition	Determine if a compound has the potential to induce or inhibit hepatic enzymes	Yes	+++
Hepatotoxicity	Determine if a compound and its metabolites have the potential to be hepatotoxic	Yes	+++
Metabolic profiling	Determine which enzymes metabolize a compound	Yes	+++
Metabolic stability	Measure the disappearance of a compound in the presence of metabolizing enzymes	Yes	++
Transporter uptake	Determine if a compound has the potential to inhibit or induce liver transporter uptake	Yes	+++
Transporter efflux	Determine if a compound has the potential to inhibit or induce liver basolateral transporter efflux	Yes	+++
Other research applications (R&D, toxicology, environmental safety)			
Environmental bioaccumulation	Evaluate bioaccumulation of drugs or chemicals in humans	No	+++
Liver disease research	Improve understanding of how the liver is involved in disease	No	+++
siRNA, basic research	Identify the effects of gene suppression on disease	No	+++

* US FDA (2017) *In Vitro* Metabolism- and Transporter-Mediated Drug-Drug Interaction Studies, Guidance for Industry.

+++ Product highly recommended for this application.

++ Product recommended for this application.

+ Our lowest recommendation, but product may still be useful for some assays.

Suspension hepatocytes	Liver microsomes	Liver S9 fractions	Other	Featured product	Cat. No.
				Gibco Human Cryopreserved Plateable Hepatocytes, Induction Qualified Also available: • Cryopreserved animal hepatocytes	HMCPIS
++	+++			Gibco Human Pooled Microsomes Also available: • Cryopreserved suspension hepatocytes	HMMCPL
+++				Gibco Human Cryopreserved Plateable Hepatocytes, Metabolism Qualified Also available: • Cryopreserved human and animal hepatocytes	HMCPMS
+++	+++	+		Gibco Human Pooled Microsomes Also available: • Animal microsomes • Human and animal cryopreserved hepatocytes • Human and animal liver S9 fractions	HMMCPL
+++				Gibco Human Cryopreserved Suspension Hepatocytes, Transporter Qualified Also available: • Human plateable hepatocytes, transporter qualified • Animal hepatocytes	HMCSTS
			ABC vesicles	Gibco Human Cryopreserved Plateable Hepatocytes, Transporter Qualified Also available: • GenoMembrane ABC inside-out vesicles • Animal plateable hepatocytes	HMCPTS
++		+++		• Animal and human cryopreserved hepatocytes	HMCPIS
+++	++			Gibco HEP10 Pooled Human Cryopreserved Hepatocytes Also available: • Animal and human cryopreserved hepatocytes • Animal and human microsomes	HMCS10
				Gibco Human Cryopreserved Plateable Hepatocytes, Transporter Qualified Also available: • Animal cryopreserved plateable hepatocytes	HMCPTS

Gibco Cryopreserved Human and Animal Hepatocytes

Gibco™ primary hepatocytes isolated from the liver are effective tools for the *in vitro* evaluation of metabolism, drug–drug interactions, hepatotoxicity, and transporter assessment. Our technicians are extensively trained in proper techniques to help ensure optimal cell health. As a result, Gibco hepatocytes have high viabilities, *in vivo*–like enzyme expression levels, and, if released as plateable cells, excellent confluencies that contribute to polarization and functioning cell–cell contacts (Figure 1). Our primary hepatocytes offer:

- Viabilities routinely >80%
- Large and multiple lots—ideal for long-term studies across sites
- Characterization for phase I and phase II drug-metabolizing activities

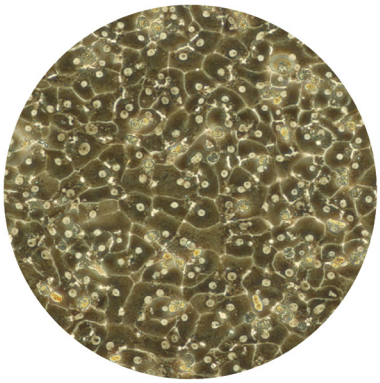
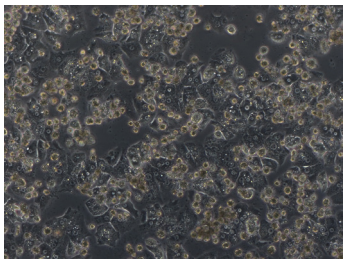


Figure 1. Confluent, healthy, sandwich-cultured human hepatocytes shown after 5 days of culture. The high level of confluency (>90%) and observable canaliculi are signs that this lot is suitable for metabolism, induction, and transporter uptake experiments.

Characterization and quality control

- **10-point cell morphology check backed by photomicrographs**—cell membrane integrity, organelle size, presence of lipid droplets, nucleus size and shape, cytosolic clarity, cell shape, level of cell debris, cell excretion products, cell–cell contacts (plateable cells only), and reestablishment of bile canalicular networks (plateable cells only)
- **Metabolic activity testing**—ECOD, 7-HCG, 7-HCS; human hepatocytes include testing for CYP1A2, CYP2B6, CYP2C8, CYP2C9, CYP2C19, CYP2D6, CYP2E1, CYP3A, and FMO
- **Application qualification tests**—attachment efficiency, monolayer confluency (Figure 2), fold induction with prototypical inducers, transporter uptake and efflux, *in situ* intrinsic clearance
- **Additional characterization data**—genotyping, optimal seeding densities (Figures 3 and 4), viability stabilities, donor demographic

Lack of monolayer integrity ("unhealthy" morphology)



- Low confluency (<70%)
- Limited cell–cell contact
- Grainy cytoplasm
- Visible cell stretching
- Severe cell flattening
- Absence of bile canaliculi

Ideal monolayer integrity ("healthy" morphology)

- High confluency (>90%)
- Good cell–cell contact
- Clear cytoplasm
- 3D configuration
- Cuboidal cell structure
- Bile canaliculi formation

Figure 2. Monolayer integrity of human hepatocytes. Representative images that demonstrate lack of monolayer integrity and ideal monolayer integrity from cryopreserved human hepatocytes isolated from separate donor tissues and cultured for multiple days. Note: An unhealthy monolayer can demonstrate confluence but lack integrity due to cell stretching and flattening.

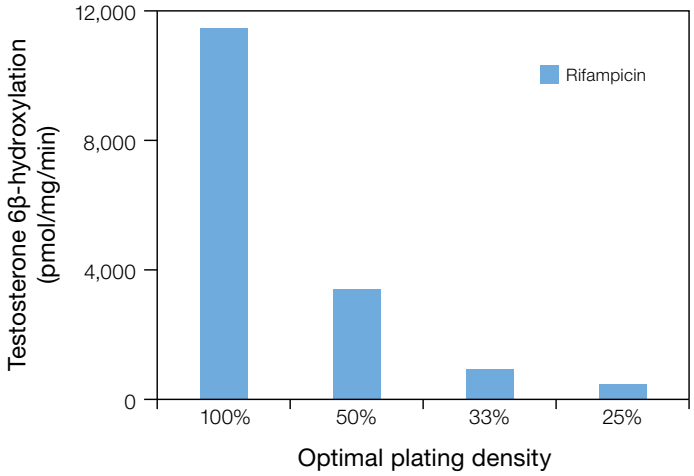


Figure 3. Seeding density effects. Induction response varies based on the level of plating density. A better response to rifampicin is seen here with an optimized plating density.

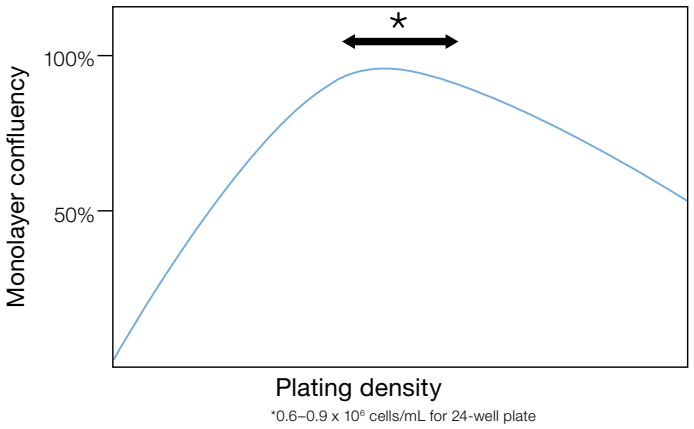


Figure 4. General relationship between plating density and monolayer confluence. For human hepatocytes, the optimal plating range to achieve maximal confluence is 0.6–0.9 x 10^6 cells/mL for a 24-well plate.

Gibco Human Cryopreserved Hepatocytes, Transporter Qualified

Gibco™ plateable and suspension hepatocytes are assessed for transporter function using uptake transporter assays. These cells:

- Contain functional membrane receptors and transporters
- Facilitate effective transporter uptake and basolateral efflux (plated) experiments
- Have stringent release specifications: ≥80% viability and ≥80% confluency (plated cells)

Measurement of transporter uptake using suspension hepatocytes

Transporter-qualified cryopreserved suspension hepatocytes are suitable for hepatic uptake studies, which typically measure the rate of appearance of substrate in cells after a relatively short incubation period, in most cases from 15 seconds to 3 minutes. Each of our transporter-qualified lots (suspension and plateable) has been functionally tested for the activities of NTCP, OATP1B3, and OATP transporter pathways using the substrates taurocholate, digoxin, and estradiol 17β glucuronide (E2-17G), as well as phase I and phase II metabolic activities. Visualization of the bile canalicular networks is attained by fluorescence microscopy using the compound 5-(6)-carboxy-2′,7′-dichlorofluorescein diacetate (CDFDA). CDFDA is a substrate of the MRP2 transporter protein and accumulates in the bile canaliculi as the cells polarize and form bile canaliculi over 3–4 days in culture (Figure 5). Transporter uptake data have been compared before and after cryopreservation with similar results, suggesting that transporter gene expression in suspension hepatocytes is not affected by cryopreservation (Figure 6).

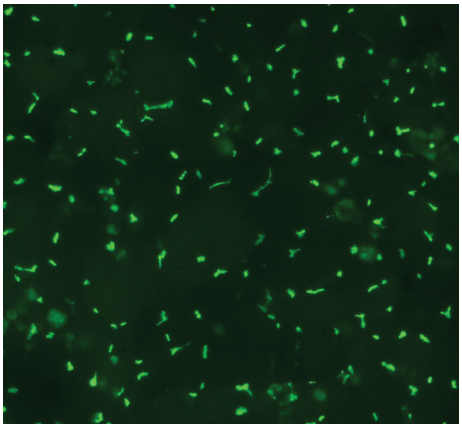


Figure 5. Visualization of functional bile canaliculi networks showing the accumulation of 5-(6)-carboxy-2′,7′-dichlorofluorescein (CDF).

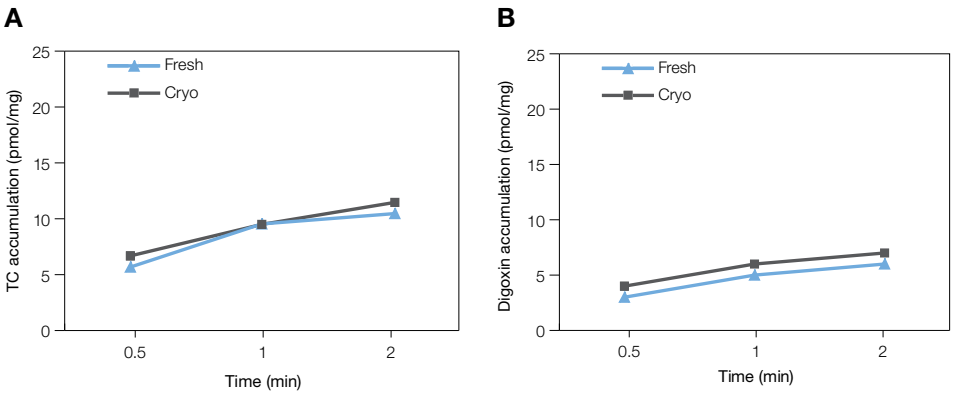


Figure 6. Comparison of human suspension hepatocyte activities before and after cryopreservation. A single lot of human hepatocytes was functionally tested for transporter uptake after 2 hours post-isolation and subsequent cryopreservation. Average accumulation of two substrates, **(A)** Na-taurocholate and **(B)** digoxin, was similar under both conditions.

Gibco Human Plateable Hepatocytes, Induction Qualified

Gibco™ plateable hepatocytes are tested with clinically relevant inducers and are suitable for use in experiments monitoring *in vitro* enzyme induction:

- Prequalified for CYP1A2, CYP2B6, and CYP3A induction
- ≥80% viability and ≥80% confluency (plated cells)
- Minimum specific activities:
  - ≥10-fold induction of CYP1A2
  - ≥5-fold induction of CYP2B6
  - ≥3-fold induction of CYP3A

Qualification of lots for use in experiments monitoring enzyme induction

Our induction-qualified hepatocytes have passed our quality tests for specific activity and mRNA levels in response to prototypical inducers. We culture our cryopreserved hepatocytes in 24-well collagen-coated plates and dose in triplicate with vehicle (0.1% DMSO), omeprazole, phenobarbital, and rifampicin for 72 hours.

Once monolayers are washed, they are incubated with substrates phenacetin, bupropion, and testosterone to determine CYP1A2, CYP2B6, and CYP3A activities, respectively (Figure 7). Fold induction of specific activity is expressed as the ratio of induced activity to vehicle activity. mRNA content is also determined by Applied Biosystems™ TaqMan® qRT-PCR analysis after 48 hours of treatment.

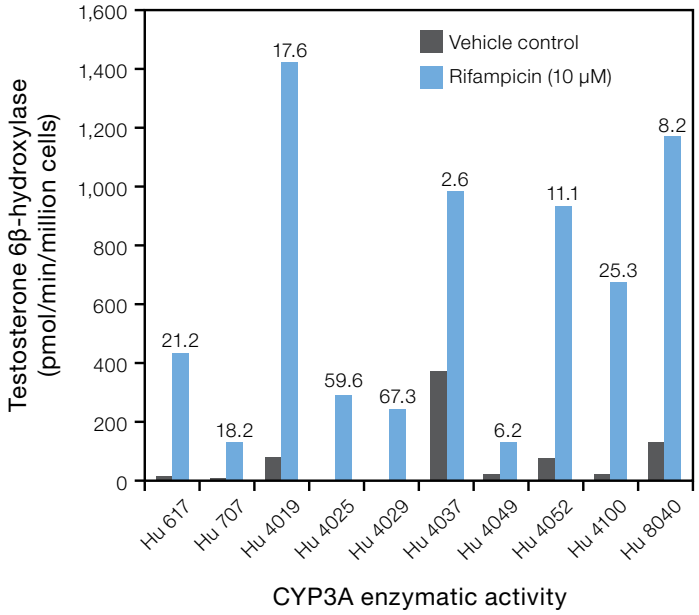


Figure 7. Testing for CYP3A fold induction. Gibco™ human cryopreserved hepatocytes are tested for response to prototypical inducers to determine suitable applications. In this example, the fold induction of CYP3A (number shown above the bar line) is calculated, illustrating inherent variability in individual lots of hepatocytes.

Gibco Human Cryopreserved Plateable Hepatocytes, Metabolism Qualified

Gibco™ plateable hepatocytes are suitable for use in applications involving metabolism of compounds:

- Useful for the assessment of intrinsic clearance (CL_{int}) of low-turnover compounds
- Prequalified according to CL_{int} of midazolam, tolbutamide, and dextromethorphan
- ≥80% viability and ≥75% attachment efficiency

Metabolic assay conditions for plated metabolism-qualified human hepatocytes

Our plated metabolism-qualified hepatocytes have been tested for the enzymatic functions of CYP3A4, CYP2C9, and CYP2D6, using the prototypical CYP450 substrates midazolam, tolbutamide, and dextromethorphan, respectively (Figure 8). Using 48-well collagen-coated plates, hepatocytes are allowed to attach prior to incubation in serum-free Gibco™ Williams Medium E, with reactions stopped with ice-cold acetonitrile. Well contents are stored at –70°C prior to analysis. The disappearance of the parent compound is monitored by LC-MS/MS and intrinsic clearance values determined by linear regression.

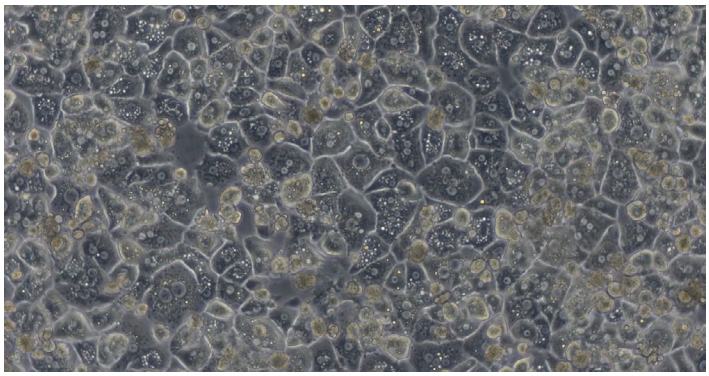


Figure 8. Cryopreserved human hepatocytes prequalified for plated metabolism (intrinsic clearance). CL_{int} (µL/1 x 10^6 cells/min) results were midazolam, 14.6; tolbutamide, 134; dextromethorphan, 7.20.

Gibco HEP10 Pooled and Single-Donor Human Cryopreserved Hepatocytes, Metabolism Qualified

Our Gibco™ HEP10™ pooled and single-donor human cryopreserved suspension hepatocytes are ideal for metabolism studies such as metabolic profiling and metabolic stability. As an industry leader in liver tissue sourcing and hepatocyte isolations, we offer one of the largest selections of single-donor and pooled lots (Figure 9). Our pooled lots are produced from normal donors with average CYP450 values, simplifying your experiments that may require the use of multiple donors. HEP10 hepatocytes are:

- Characterized for CYP1A2, CYP2B6, CYP2C8, CYP2C9, CYP2C19, CYP2D6, CYP3A, ECOD, 7-HCG, and 7-HCS activities
- Pooled as high-quality lots with batch sizes of >500 vials
- Available as specialty lots, including low CYP2D6 metabolizers

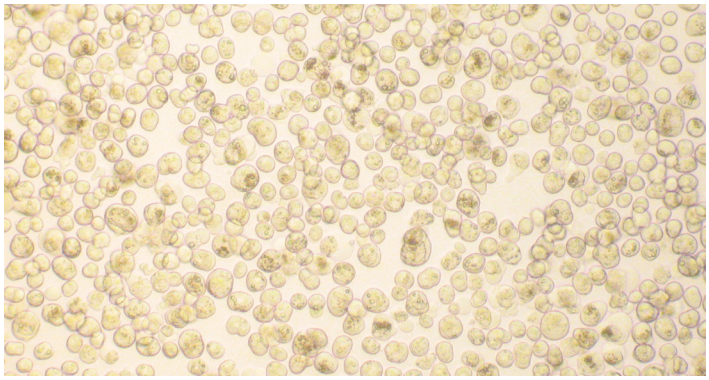


Figure 9. Human hepatocytes qualified for suspension metabolism applications.

Gibco Human Spheroid-Qualified Hepatocytes

As research methods evolve, investigators must adapt their tools to enable interrogation of more complex and physiologically relevant models. Gibco™ spheroid-qualified hepatocytes are off-the-shelf cryopreserved cells for the generation of 3D cellular models. Hepatic spheroid cultures maintain cellular function for at least 21 days and demonstrate marked increases in albumin protein production and CYP3A4 enzyme activity (Figures 12 and 13).

Why use Gibco Human Spheroid-Qualified Hepatocytes?

- **Improved culture longevity**—up to 21 days, more than 4x longer than traditional 2D monolayer culture
- **Design your own schedule**—cells are ready for plating when you are
- **Interrogate pathways of interest**—applicable for toxicity, metabolism, or disease modeling

Find out more about how our scientists are using 3D hepatic models for drug metabolism testing and gene expression studies at **thermofisher.com/3dheps**

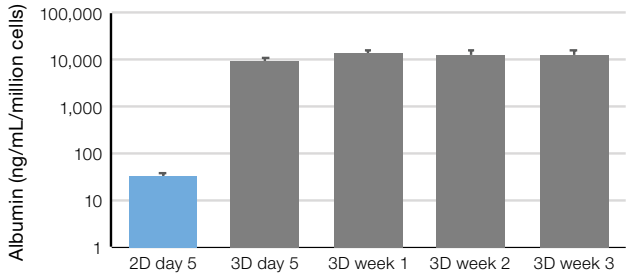


Figure 10. Albumin secretion in 2D and 3D spheroid hepatic cultures. The concentration of albumin secreted is normalized to the total number of cells per well.

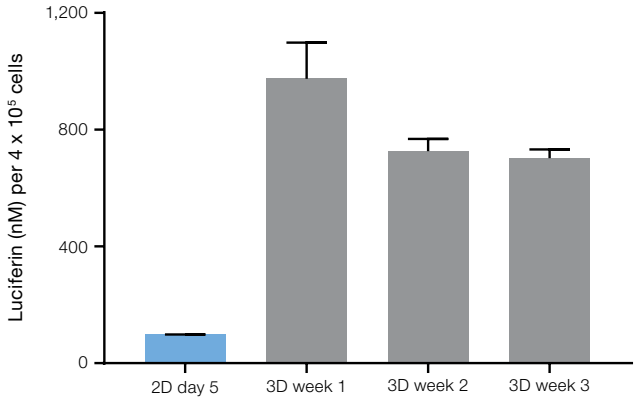


Figure 11. CYP3A4 activity in 2D and 3D spheroid hepatic cultures. CYP3A4 activity was measured using the Promega™ P450-Glo™ CYP3A4 Assay with Luciferin-IPA. CYP3A4 activity was found to be significantly higher in the 3D spheroids than in the 2D culture. The data presented are the mean ± SEM (n = 3 for the 2D culture, n = 8 for the 3D spheroids).

Gibco Activated Human Myofibroblastic Hepatic Stellate Cells

Human hepatic stellate cells (HSCs) are the major contributor to collagen deposition following liver damage, where hepatic stellate cells activate to a phenotype characterized by increased proliferation, motility, contractility, and synthesis of extracellular matrix components that result in progressive liver fibrosis. Activated HSCs are ideal for building hepatic co-cultures with primary hepatocytes for *in vitro* study of diseases such as fibrosis (Figure 14).

Find out more about how our scientists are using stellate cells in 2D and 3D co-culture systems to model better liver fibrogenic responses at **thermofisher.com/stellate**

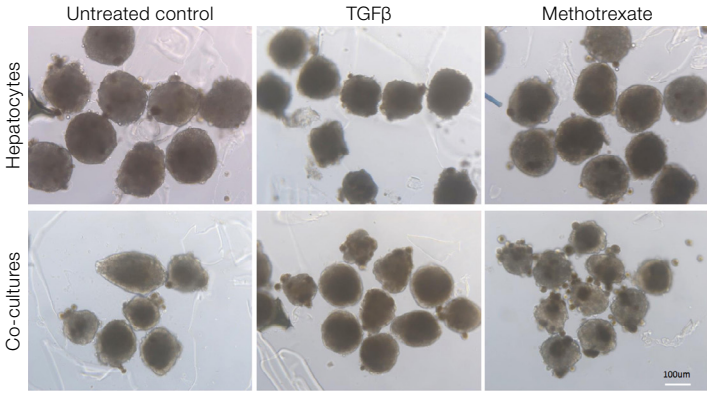


Figure 12. Morphology of 3D spheroids of hepatocytes alone or co-cultures of hepatocytes and myofibroblastic HSCs, following treatment with TGFβ and methotrexate for 14 days. Phenotypic morphology shows differential density of hepatocyte spheroids that appear more uniform and denser than co-culture spheroids. Smaller vesicle-like structures are predominantly visible in co-cultures following treatment with methotrexate.

Gibco Cryopreserved Kupffer Cells

Kupffer cells provide improved physiological modeling

Growing evidence shows that under both normal and pathological conditions, many hepatocyte functions are regulated by substances released from neighboring nonparenchymal cells (NPCs). These cells, particularly Kupffer cells, play an important role in the modulation of xenobiotic metabolism in the liver. Kupffer cells secrete potent mediators of the inflammatory response that controls liver inflammation. These cytokine mediators control hepatocyte metabolic rates through direct interactions with phase I and phase II enzymes.

Co-cultured Kupffer cell and hepatocytes can self-assemble within 72 hours of treatment with pro-inflammatory cytokines or lipopolysaccharides (LPS), and can function by effectively modulating P450 expression, thus giving a more physiologically relevant result (Figures 10 and 11). Studies indicate that co-cultures of hepatocytes with NPCs better represent both normal liver physiology and disease states. Cryopreserved purified human and rat Kupffer cells are a convenient way to produce hepatocyte and Kupffer cell co-cultures for the study of various hepatic functions. With Gibco™ Cryopreserved Kupffer Cells, you get:

- High viability and purity, routinely >90%
- Response to activation with LPS
- Minimum 1 million viable cells per vial
- Hepatocyte co-culture protocols

Find out more at **thermofisher.com/kupffer**

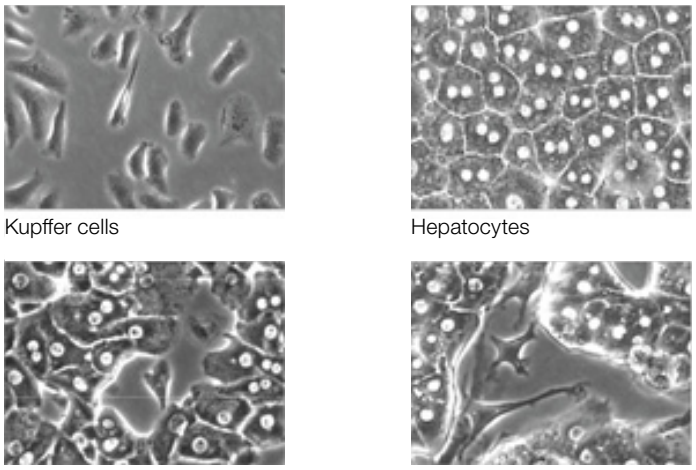


Figure 13. Co-cultures modeling normal and inflamed liver states. These models enable researchers to study the interactions between hepatocytes and Kupffer cells during liver inflammation.

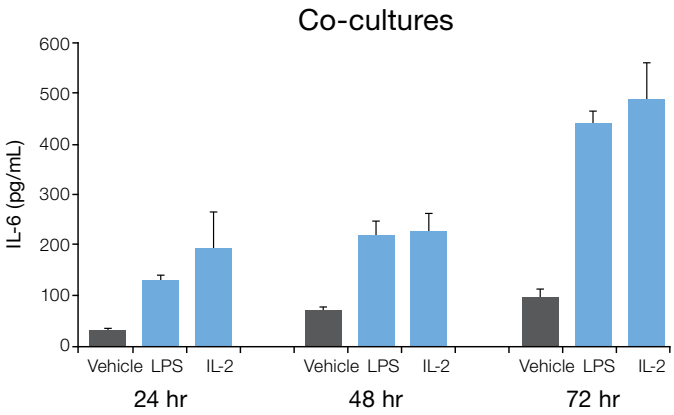


Figure 14. IL-6 production in co-cultures of Kupffer cells and hepatocytes after LPS and IL-2 stimulation for 24, 48, and 72 hours. Note that IL-6 is significantly up-regulated in co-cultures at all time points. This suggests cellular self-assembly between Kupffer cells and hepatocytes that synergistically allows those cells to function together during resolution of inflammation.

Gibco Animal Cryopreserved Plateable and Suspension Hepatocytes

Our Gibco™ animal cryopreserved hepatocytes are prepared using the same careful isolation and cryopreservation techniques as our human lots. We routinely isolate hepatocytes from mouse, rat, dog, and nonhuman primate. Viabilities for these cells are routinely >80%. Characterization methods include ECOD, 7-HCG, and 7-HCS for phase I and phase II enzyme activities. We also closely monitor cell morphology, attachment efficiency, monolayer confluency (plateable cells), and viability stability over time.

Gibco HepaRG Cells

The Gibco™ HepaRG™ cell line is an immortalized hepatic cell line that retains many characteristics of primary human hepatocytes. HepaRG cells are terminally differentiated and provided in a convenient cryopreserved format. For scientists who need reproducible metabolism data, HepaRG cells are an *in vitro* tool that provides results in a metabolically complete and scalable system. These cells enable you to:

- Obtain biologically relevant results from a metabolically complete system
- Assess the drug–drug interaction potential of your compound
- Experience reproducible results from a single population of cells

Biologically relevant

HepaRG cells exhibit many characteristics of primary human hepatocytes, including morphology, expression of key metabolic enzymes, expression of nuclear receptors, and drug transporters. Unlike HepG2 and Fa2N-4 cells, HepaRG cells have high P450 activity and complete expression of all nuclear receptors.

Predict metabolism-based drug–drug interactions

HepaRG cells respond to prototypical P450 inducers and inhibitors to the same extent as primary hepatocytes, allowing HepaRG cells to be used to evaluate potential drug–drug interactions.

Consistent performance

HepaRG cells are essentially a single donor. This enables users to obtain physiologically relevant results for metabolism-based drug–drug interactions without the concern of donor variability and limited lot sizes that come with relying on donor tissue. Note: The cells we provide are terminally differentiated.

HepaRG cells in 3D culture

Find out more about how our scientists are using HepaRG cells in 3D culture and review our newest scientific posters on liver modeling at **thermofisher.com/admetox**

Cell culture reagents

Gibco™ HepExtend™ Supplement (50X)

HepExtend Supplement (50X) has been optimized for use with cryopreserved primary hepatocytes to improve cell viability, function, and number of days in culture. This enables researchers to perform metabolic and toxicity experiments not achievable using standard culture conditions (Figures 15 and 16). Simply add HepExtend Supplement to standard Williams Medium E and use with your current hepatocyte culturing methods and instrumentation. The supplement does not contain any small molecules or fetal bovine serum, components that are known to interfere with primary hepatocyte function.

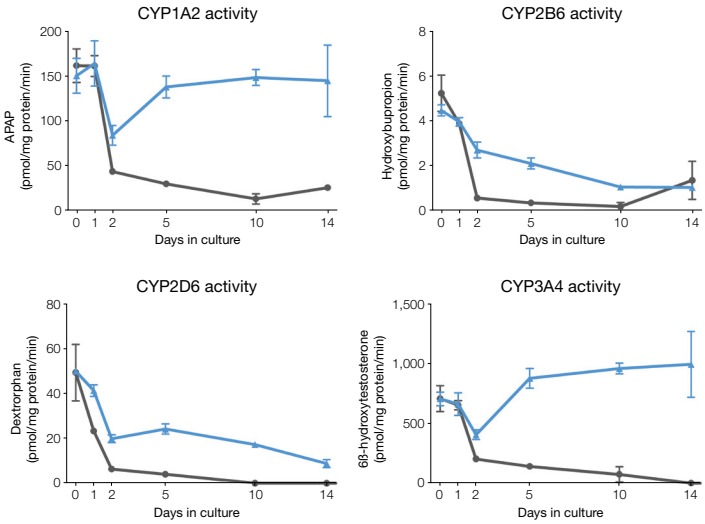


Figure 15. Cytochrome P450 specific activity over the course of a 14-day culture. HepExtend Supplement cultures enhance cytochrome P450 activity compared to standard Williams Medium E.

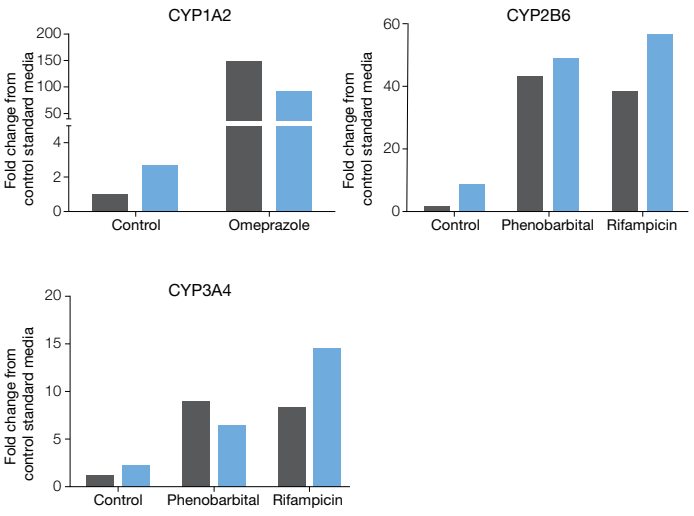


Figure 16. Human hepatocytes cultured in HepExtend Supplement respond to inducers for Ah receptor, PXR, and CAR.

- **Enabling**—designed to provide maximum cell viability and cell health when culturing primary plateable hepatocytes
- **Extended cell survival**—days in culture can be extended to >10 days while maintaining normal cell morphology and bile canaliculi
- **Consistent**—manufactured in a cGMP-compliant facility using the highest-quality materials

Thawing media

Gibco™ Hepatocyte Thaw Medium (HTM) and Gibco™ Cryopreserved Hepatocyte Recovery Medium (CHRM) are proprietary formulations designed to enhance the recovery of viable hepatocytes while removing cryoprotectant after cell cryopreservation. When used appropriately, they have proven to result in healthier hepatocytes with consistently higher viability (Figure 17). Both are easy to use: simply add one vial of thawed hepatocytes to the conical tube, centrifuge briefly, remove the medium, and gently resuspend the cell pellet.

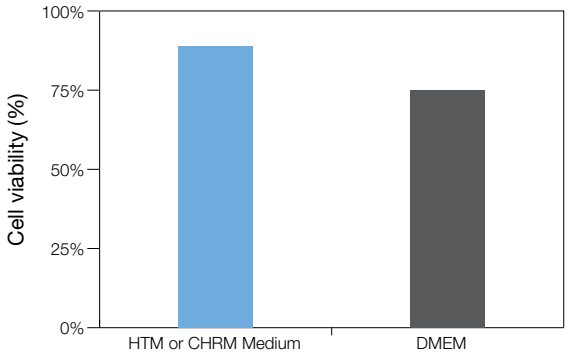


Figure 17. Typical results of HTM or CHRM vs. DMEM in the ability to enhance hepatocyte viability post-cryopreservation.

Gibco Williams Medium E

We recommend a phenol red–free Williams Medium E for hepatocyte research involving LC-MS/MS analysis.

Gibco™ Hepatocyte Plating Supplement Pack

The Hepatocyte Plating Supplement Pack contains prequalified fetal bovine serum, dexamethasone, and a cocktail solution of penicillin–streptomycin, bovine insulin, GlutaMAX™ medium, and HEPES to supplement up to 500 mL of Williams Medium E without phenol red, or a suitable alternative basal medium, for the purpose of plating fresh or cryopreserved hepatocytes.

Gibco™ Hepatocyte Maintenance Supplement Pack

The Hepatocyte Maintenance Supplement Pack contains dexamethasone and a cocktail solution of penicillin–streptomycin, ITS+ (insulin, transferrin, selenium complex, BSA, and linoleic acid), GlutaMAX medium, and HEPES to supplement up to 500 mL of Williams Medium E without phenol red, or a suitable alternative basal medium, for the purpose of incubating hepatocytes in suspension or plated cultures.

Gibco™ Collagen I, Rat Tail

Collagen is the most widely used extracellular matrix (ECM) protein for cell culture, facilitating cell attachment and differentiation. In addition to our 5 mg/mL Collagen I solution, we also offer Collagen I precoated 6-, 24-, and 96-well plates for your hepatocyte experiments.

Gibco™ Geltrex™ LDEV-Free Reduced Growth Factor Basement Membrane Matrix

Geltrex matrix is a soluble form of reduced growth factor (RGF) basement membrane extract (BME) purified from continuous sheets of specialized extracellular matrix that form an interface between Engelbreth-Holm-Swarm (EHS) tumor cells. The major components of Geltrex matrix include laminin, collagen IV, entactin, and heparin sulfate proteoglycan, which provide the foundation for 3D culture studies.

3D culture and analysis

Organoids and spheroids show great potential in many applications, including drug discovery, toxicology, and disease modeling. These 3D cell models offer opportunities to better understand complex biology in a physiologically relevant context. As advances in culturing organoids and spheroids become more common, the need for cell culture guidance and product recommendations is becoming more prevalent. As a leader in cell culture products, we want to partner with you to facilitate the transition from 2D monolayer cultures to 3D cell models.

Find out more about how our scientists are using 3D culture and learn how you can accelerate you current projects at **thermofisher.com/3dmodels**

Gibco Liver Microsomes and S9 Fractions

Subcellular fractions derived from the endoplasmic reticulum of liver contain a variety of metabolic enzymes for assessing the *in vitro* metabolism of drug candidates (Table 2) and are suitable for a variety of experiments:

- Metabolite characterization
- Cytochrome P450 inhibition studies and phenotyping
- Metabolic stability

We provide Gibco™ Liver Microsomes from a variety of human and other toxicology research species. Each product contains an average representative pool of donors.

Human microsome pools are fully characterized (K_m and V_{max}) according to GLP standards for major cytochrome P450 activities and selected phase II enzymes using FDA-recommended substrates (Table 3). We have:

- Large pooled lots for reproducible, long-term studies
- Specialty human pools based on age, BMI, high CYP3A4, or high CYP2D6
- Other subcellular fractions available, including S9 and cytosolic fractions

Gibco™ S9 Fractions are available from the following species:

- Human (single-donor, specialty pools, population pools)
- Rat (Sprague-Dawley)
- Mouse (CD-1)
- Dog (beagle)
- Nonhuman primate (cynomolgus)

Find out more at **thermofisher.com/microsomes**

Table 2. Metabolic enzymes found in liver subcellular fractions.

Metabolic enzyme	Liver microsomes	Liver S9 fractions	Liver cytosol
Aldehyde oxidase		•	•
Cytochrome P450 (CYP)	•	•	
Flavin monooxygenases (FMO)	•	•	
Glutathione transferase (GST)		•	
Monoamine oxidase (MAO)		•	
Sulfotransferases (SULT)		•	•
Uridine diphosphate (UDP)-glucuronosyltransferase (UGT)	•	•	

Table 3. Kinetic parameters for the current lot of Gibco™ Human Liver Microsomes, 50-donor pool.

Isoform	Metabolite	K_m (µM)	V_{max} (nmol/min/mg)
CYP1A2	Acetaminophen	78	0.73
CYP2A6	7-Hydroxycoumarin	1.1	0.53
CYP2B6	Hydroxybupropion	64	0.29
CYP2C8	6α-Hydroxypaclitaxel	5.5	0.15
CYP2C9	Hydroxytolbutamide	220	0.19
CYP2C19	4′-Hydroxymephenytoin	34	0.031
CYP2D6	Dextrorphan	3.2	0.13
CYP2E1	6-Hydroxychlorzoxazone	70	1.4
CYP3A4	6β-Hydroxytestosterone	19	4.0
CYP3A4	1′-Hydroxymidazolam	1.6	1.1

Cytochrome P450 BACULOSOMES Plus reagents and Vivid CYP450 screening kits

Invitrogen™ CYP450 BACULOSOMES™ Plus reagents are microsomes prepared from insect cells infected with a recombinant baculovirus containing a human CYP450 isozyme, as well as human cytochrome P450 reductase. Invitrogen™ Vivid™ CYP450 screening kits are high-throughput, fluorescence-based assays for detection of enzyme–drug interactions and CYP450 inhibition. CYP450 BACULOSOMES Plus reagents and Vivid CYP450 screening kits offer:

- A single human P450 isozyme for detailed drug metabolism studies
- An easy three-step procedure, “mix and read” format; reactions performed at room temperature or 37°C
- High signal-to-background ratio, broad dynamic range
- Compatibility with multiple assay formats from 96-well to 1,536-well

Single overexpressed human CYP450 isozyme

CYP450 BACULOSOMES Plus reagents offer a distinct advantage over human liver microsomes in that only one CYP450 isozyme is expressed, thereby preventing metabolism by other CYP450 isozymes or other classes of drug-metabolizing enzymes.

Unique Vivid reagents for bright fluorescent signals and low background

Invitrogen™ Vivid™ fluorogenic substrates are blocked dyes that yield minimal fluorescence signal until cleaved or hydroxylated. Oxidation at either of two potential sites releases the highly fluorescent product. They have superior fluorescence, solubility, and kinetic properties compared to conventional fluorogenic probes. This results in higher sensitivity, greater signal-to-noise ratio, and better assay reproducibility. The choice of substrate yields a product that emits blue, green, red, or cyan fluorescence.

Flexible assay formats for optimized results

The sensitivity of the Vivid CYP450 assays allows detection of weak inhibitors and miniaturization to as little as 2 µL per reaction. Assays may be set up in kinetic mode or in endpoint mode to facilitate multi-plate screening. Assays may be performed at room temperature or 37°C.

For full listings of CYP450 enzymes and Vivid kits, visit **thermofisher.com/admetox**

Transporter products

Let us help you evaluate your compound's transporter interactions. Our comprehensive offering of transporter-qualified hepatocytes, vesicles, kits, and transporter cells can help you in the prediction of bioavailability and clearance of your compound, as well as the potential for drug–drug interactions and toxicities.

ATP-binding cassette (ABC) transporter vesicles and kits

Gibco™ ABC transporter vesicles are easy-to-use, efficient reagents for early assessment of a drug candidate's substrate and drug interaction potential. These vesicles, manufactured by GenoMembrane Co., are prepared by advanced methodologies using plasma membrane purified from an insect cell system (Sf9 cells transfected with baculovirus) that overexpresses ABC transporters. These "inside-out" vesicles provide high levels of transporter activity with low background, giving you a clear signal if your compound is a substrate or inhibitor of a specific efflux transporter. ABC transporter vesicles are prepared from ABC transporter membranes for use in vesicular transport assays. While ABC transporters typically mediate the efflux of substrates from cells, transporters expressed on these inside-out vesicles import substrates into the vesicles. It is therefore possible to quantitatively evaluate transport activity for your compound by determining the amount incorporated into the vesicles.

- Provides easy, targeted assessment of efflux transporters
- Have high signal-to-noise ratios
- Are available for a wide selection of species and transporters

TRANSiPORT human SLC transporter cells

Gibco™ TRANSiPORT human SLC transporter cells, manufactured by GenoMembrane Co., are HEK293 cells that transiently overexpress a solute carrier (SLC) transporter protein. Solute carriers are one of two superfamilies of membrane transporters that can affect pharmacokinetics and drug exposure by governing the transport of solutes into and out of cells. Membrane transporters are found in hepatic cells, kidney proximal tubule cells, intestinal cells, and brain cells.

- Assess potential for transporter-mediated drug metabolism
- Easy-to-use format
- Obtain high-quality results with a high signal-to-noise ratio

Ordering information

Product	Quantity	Cat. No.
ABC transporter vesicles and kits		
Human BCRP Vesicles	0.5 mL	GM0008
Human BSEP Vesicles	0.5 mL	GM0005
Human MDR1 Vesicles	0.5 mL	GM0015
Human MRP1 Vesicles	0.5 mL	GM0010
Human MRP2 Vesicles	0.5 mL	GM0001
Human MRP3 Vesicles	0.5 mL	GM0021
Human MRP4 Vesicles	0.5 mL	GM0012
Human MRP8 Vesicles	0.5 mL	GM0013
Dog BSEP Vesicles	0.5 mL	GM0019
Dog MRP2 Vesicles	0.5 mL	GM0014
Monkey MRP2 Vesicles	0.5 mL	GM0018
Rat BCRP Vesicles	0.5 mL	GM0007
Rat BSEP Vesicles	0.5 mL	GM0006
Rat MRP2 Vesicles	0.5 mL	GM0002
BSEP Vesicular Transport Assay Reagent Set	100 assays	GM3001
MDR1 Vesicular Transport Assay Reagent Set	100 assays	GM3030
MRPs-BCRP Vesicular Transport Assay Reagent Set	100 assays	GM3010
Control Vesicles	0.5 mL	GM0003

Note: One vial of vesicle product can perform 50 vesicular transport assays.

TRANSiPORT human SLC transporter cells		
Mock SLC Transporter Cells	8–10 x 10^6 cells	GM1001
Human OATP1B1 SLC Transporter Cells	8–10 x 10^6 cells	GM1002
Human OAT1 SLC Transporter Cells	8–10 x 10^6 cells	GM1003
Human OAT3 SLC Transporter Cells	8–10 x 10^6 cells	GM1004
Human OCT2 SLC Transporter Cells	8–10 x 10^6 cells	GM1005
Human OATP1B3 SLC Transporter Cells	8–10 x 10^6 cells	GM1006
Human OATP2B1 SLC Transporter Cells	8–10 x 10^6 cells	GM1007
Human OCT1 SLC Transporter Cells	8–10 x 10^6 cells	GM1008
Human NTCP SLC Transporter Cells	8–10 x 10^6 cells	GM1013
Human MATE1 SLC Transporter Cells	8–10 x 10^6 cells	GM1014
Human MATE2K SLC Transporter Cells	8–10 x 10^6 cells	GM1015
Human OATP1A2 SLC Transporter Cells	8–10 x 10^6 cells	GM1016
Mock OATP1A2 SLC Transporter Cells	8–10 x 10^6 cells	GM1017

Nuclear receptors

Nuclear receptors are a superfamily of transcription factors that can mediate transcription regulation in ADME-relevant genes. To facilitate your drug discovery efforts through the study of nuclear receptors, we have developed highly sensitive Invitrogen™ LanthaScreen™ Nuclear Receptor Competitive Binding Assays, suitable for high-throughput screening.

Our LanthaScreen Nuclear Receptor Competitive Binding Assays (Table 4) contain all the needed reagents to conduct compound affinity testing:

- Purified nuclear receptor protein with ligand-binding domain (LBD)
- A proprietary fluorescent Invitrogen™ Fluormone™ ligand
- An optimized proprietary buffer system
- Terbium-labeled anti-GST antibody

The nuclear receptor (NR) protein is indirectly labeled with terbium via the anti-GST antibody bound to the GST tag on the receptor. When this labeled protein binds to the Fluormone ligand, a high TR-FRET ratio will result. If a test compound that binds to the NR protein is added to the reaction, the test compound will compete with the Fluormone ligand, resulting in a lower TR-FRET ratio. Since the change in TR-FRET ratio only occurs in the presence of a competitor, it can be used as a convenient and accurate indicator of the relative affinity of the test compound for the NR (Figure 18).

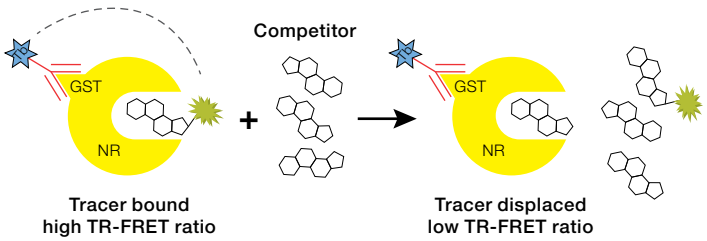


Figure 18. Schematic of a LanthaScreen nuclear receptor assay.

Table 4. Selection guide for LanthaScreen Nuclear Receptor Competitive Binding Assays.

Target	Alias	Accession number	LanthaScreen assay
Estrogen receptor alpha	ER Alpha, ERA, ESR1, NR3A1	NP_000116.2	A15887
Estrogen receptor beta	ER Beta, ERB, ESR2, NR3A2	NP_001428.1	A15895
Glucocorticoid receptor	GR, GCCR, GCR, NR3C1	NP_000167	A15901
Peroxisome proliferator-activated receptor alpha	PPAR Alpha, PPARA, NR1C1	NP_005027	PV4892
Peroxisome proliferator-activated receptor delta	PPAR Delta, PPARD, NR1C2	NP_006229	PV4893
Peroxisome proliferator-activated receptor gamma	PPAR Gamma, PPARG, NR1C3	NP_005028	PV4894
Pregnane X receptor	PXR(SXR), NR1I2	NP_003880.3	PV4839

gibco

ADME/Tox support

Protocols for ADME/Tox products

- Cell counting of primary hepatocytes using trypan blue exclusion analysis
- Metabolic stability using suspension and plated hepatocytes
- Induction potential in plated hepatocytes
- Co-culture of hepatocytes and Kupffer cells
- *In vitro* assessment of transporter-mediated uptake in plated cryopreserved hepatocytes

Need assistance selecting a product?

Our product specialists are available to help you with product and lot selection, usage protocols, and orders.

Americas and Asia Pacific—orders and technical support
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Toll-free Tel: +1 866 952 3559
Email: hepaticproducts@thermofisher.com

Europe—orders and technical support
Tel: +44 (0) 141 814 5900
Email: hepaticproducts@thermofisher.com

Japan—orders and technical support
Tel: +81 3 6832 6980
Email: JPCS@thermofisher.com

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