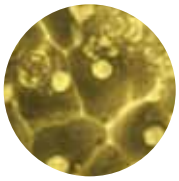
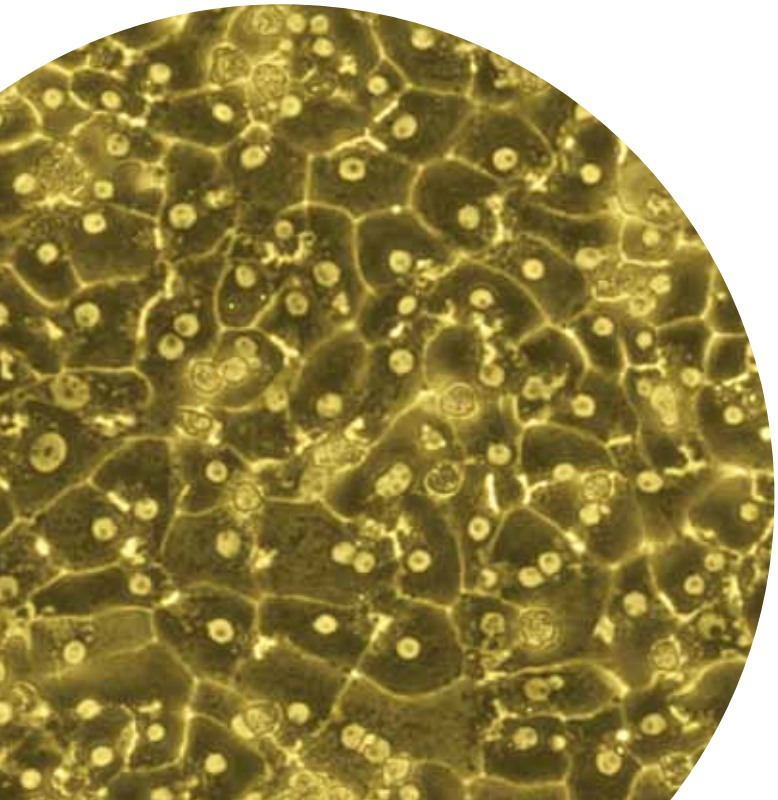
Drug development



No standard like a gold standard

Invitrogen™ hepatic biology products and services



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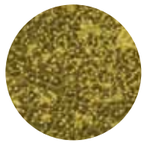

Drug development

Invitrogen hepatic biology 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:

- Cytochrome P450 (P450) inhibition and enzyme induction
- Metabolic profiling and stability
- Transporter applications

At Invitrogen, we strive to offer you industry-leading hepatic products that provide physiologically relevant results, so you can make the right decisions in relation 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 enzyme induction, P450 inhibition, hepatic transport and other metabolism-related research fields. Supplying you with the right tools and technical support is our goal.

Complete selection of *in vitro* hepatic cell products:

- Cryopreserved primary hepatocytes
- Freshly isolated primary hepatocytes (Americas and Europe)
- Hepatic microsomes
- Hepatic S9 and cytosol fractions
- Culture medium

Our comprehensive offering includes hepatocytes and subcellular fractions isolated from human, rat, mouse, dog, rabbit, nonhuman primate, trout, and other species upon request. Our products are prequalified for particular research applications, and most are supplied as pooled or single donors, often in large lot sizes to facilitate multiple and multi-site experiments.

GIBCO® quality—every step of the way

As hepatic scientists in a company that also offers *in vitro* ADME screening and development services, 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 scientific research and development
- Hepatic product specialists to lend technical support

Ordering and technical support for ADME/Tox products and services

We have dedicated staff to support our line of ADME/Tox products and services—from pre-purchase inquiries, to shipment of your order, though troubleshooting of experiments. Contact Invitrogen's ADME/Tox Product Support to:

- Place and track orders
- Get assistance with protocols
- Select hepatic products or particular lots

Americas, Middle East, and Asia Pacific (excluding Japan)

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Phone (toll): +1 919 237 4679 (Toll)
Phone (U.S. toll free): +1 866 952 3559 (U.S. Toll-free)
Fax (toll): +1 919 237 4600 (Toll)
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Email: hepaticproducts@invitrogen.com
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Email: jpinfo@invitrogen.com
Phone: +81 3 5566 6160
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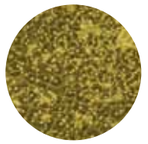
Address: 4-5-4 Hatchobori, Chuo-ku Tokyo 1040032 Japan



Drug development

Applications table

Application	Purpose	FDA guidance*	Plateable hepatocytes	Suspension hepatocytes	Liver micro-somes	Liver S9 fractions	Other	Cat. No.
Drug development research (ADME, DMPK, toxicology)								
Enzyme induction	Determine if a compound has the potential to induce hepatic enzymes	Yes	+++					→ HMCPIS and HMCPIT, GIBCO® Human Cryopreserved Plateable Hepatocytes, Induction Qualified **Also available:** → Fresh plateable human hepatocytes → Cryopreserved and fresh plateable animal hepatocytes
Enzyme inhibition	Determine if a compound has the potential to inhibit hepatic enzymes	Yes		++	+++			→ HMMCPL, GIBCO® Human Pooled Microsomes **Also available:** → Cryopreserved suspension hepatocytes
Hepatotoxicity	Determine if a compound and its metabolites have the potential to be hepatotoxic	Yes	+++	+++				→ HMCPMS and HMCPML, GIBCO® Human Cryopreserved Plateable Hepatocytes, Metabolism Qualified **Also available:** → Fresh and cryopreserved human and animal hepatocytes
Metabolic profiling	Determine which enzymes metabolize a compound	Yes		+++	+++	+		→ HMMCPL, GIBCO® Human Pooled Microsomes **Also available:** → Animal microsomes → Human and animal, fresh and cryopreserved hepatocytes → Human and animal S9
Metabolic stability	Measure the disappearance of a compound in the presence of metabolizing enzymes	Yes	++	+++	+++	+		→ HMMCPL, GIBCO® Human Pooled Microsomes **Also available:** → Animal microsomes → Human and animal, fresh and cryopreserved hepatocytes → Human and animal S9
Reaction phenotyping (metabolic identification)	Identify the metabolites formed from a compound when exposed to metabolizing enzymes	Yes	++		+++		Recombinant P450 enzymes	→ HMMCSD, GIBCO® Human Microsomes, Single Donor **Also available:** → VIVID® recombinant P450s
Transporter uptake	Determine if a compound has the potential to inhibit or induce liver transporter uptake	No	+++	+++				→ HMCSTS and HMCSTL, GIBCO® Human Cryopreserved Suspension Hepatocytes, Transporter Qualified **Also available:** → Human Plateable Hepatocytes, Transporter Qualified → Animal hepatocytes
Transporter efflux	Determine if a compound has the potential to inhibit or induce liver basolateral transporter efflux	No	+++				ABC Vesicles, ABC Membranes	→ HMCPTS and HMCPTL, GIBCO® Human Cryopreserved Plateable Hepatocytes, Transporter Qualified **Also available:** → GenoMembrane™ ABC inside-out vesicles and membranes → Animal plateable hepatocytes
Other research applications (R&D, toxicology, environmental safety)								
Environmental bioaccumulation	Evaluate bioaccumulation of drugs or chemicals in humans and fish	No	+++	++		+++		→ TRS9PL, GIBCO® Fish (Rainbow Trout) S9 Fractions **Also available:** → Animal and human, fresh and cryopreserved hepatocytes
Liver disease research	Improve understanding of how the liver is involved in disease	No	+++	+++	++			→ HMCS1S, HMCS1L, HMCS2S, and HMCS2L, GIBCO® Human Cryopreserved Suspension Hepatocytes, Metabolism Qualified **Also available:** → Animal and human, fresh and cryopreserved, hepatocytes → Animal and human microsomes
siRNA, basic research	Identify the effects of gene suppression on disease	No	+++					→ HMCPTS or HMCPTL, GIBCO® Human Cryopreserved Plateable Hepatocytes, Transporter Qualified **Also available:** → Human fresh plateable hepatocytes → Animal cryopreserved plateable hepatocytes

* Drug Interaction Studies-Study Design, Data Analysis, and Implications for Dosing and Labeling, Draft Guidance for Industry, US FDA, September 2006

GIBCO® cryopreserved human and animal hepatocytes

Primary hepatocytes isolated from the liver are effective tools for the *in vitro* evaluation of metabolism, drug–drug interactions, and hepatotoxicity, as well as transporter assessment. At Invitrogen, our hepatocyte isolationists are extensively trained in proper techniques to 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).

- Extensive selection of lots
- Viabilities routinely >80%
- Characterized for phase I and phase II drug metabolizing enzyme activities
- Multiple large lots available—ideal for long-term studies across sites

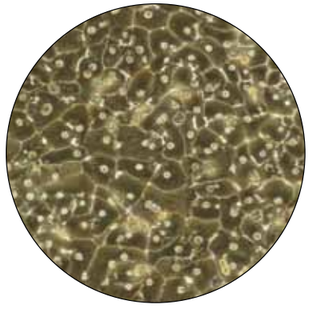
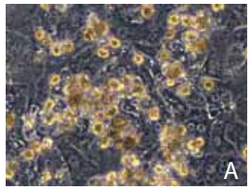
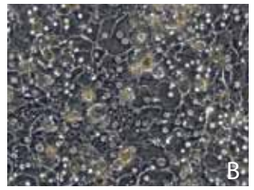


Figure 1. Confluent, healthy sandwich-cultured human hepatocytes shown after 5 days of culture. The high level of confluency (>90%) and observable canaliculi are signs that this lot is suitable for metabolism, induction, and transporter uptake experiments.

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 CYP1A2, CYP2B6, CYP2C8, CYP2C9, CYP2C19, CYP2D6, CYP2E1, CYP3A, 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 demographics

Lack of monolayer integrity ("unhealthy" morphology)	Ideal monolayer integrity ("healthy" morphology)
	
→ Low confluency (<70%)*	→ High confluency (>90%)
→ Limited cell–cell contact	→ Good cell–cell contact
→ Grainy cytoplasm	→ Clear cytoplasm
→ Visible cell stretching (e.g., fibroblast-like)	→ 3-dimensional configuration
	→ Cuboidal cell structure
→ Severe cell flattening	→ Bile canaliculi formation
→ Absence of bile canaliculi	

*** Note**: An unhealthy monolayer can demonstrate confluence but lack integrity due to cell stretching and flattening

Figure 2. Representative images that demonstrate (A) the lack of monolayer integrity and (B) ideal monolayer integrity of cryopreserved human hepatocytes isolated from separate donor tissues and cultured for multiple days.

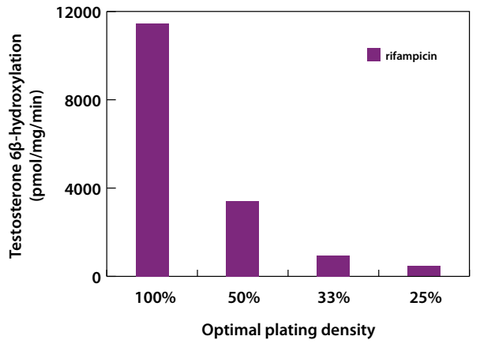


Figure 3. Seeding density effects. Induction response varies based on the plating density. A better response to rifampicin is obtained when the plating density is optimized. CYP3A4 activity was measured by testosterone 6β-hydroxylation.

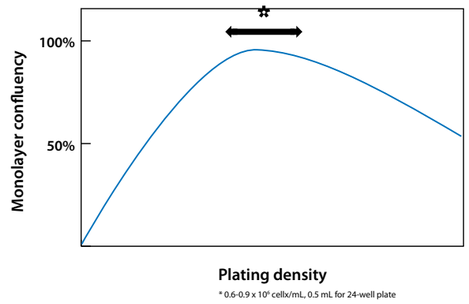


Figure 4. General relationship between plating density and monolayer confluence. For human hepatocytes, the optimal plating range to achieve maximal confluence is 0.7–0.9 x 10^6 cells/mL for a 24-well plate.

GIBCO® Human Cryopreserved Hepatocytes, Transporter Qualified

- Contain functional membrane receptors and transporters
- Facilitate effective transporter uptake and basolateral efflux (plated) experiments
- Have stringent release specs: ≥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 between 15 seconds and 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). They have also been tested for 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 of suspension hepatocytes is not affected by cryopreservation (Figure 6).

Note: For studies using B-CLEAR® technology, please refer to our B-CLEAR® Hepatic Transport Kits. GIBCO® Human Cryopreserved Hepatocytes, Transporter Qualified, are not prequalified for use with B-CLEAR® reagents and their purchase conveys no rights to practice the B-CLEAR® technology.

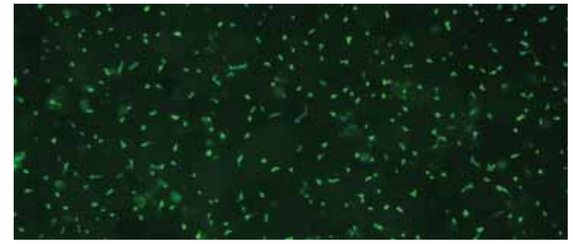


Figure 5. Visualization of functional bile canaliculi networks showing the accumulation of 5-(6)-carboxy-2',7'-dichlorofluorescein (CDF).

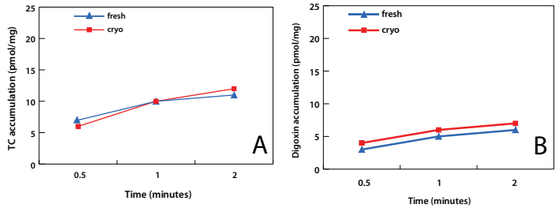


Figure 6. Comparison of human suspension hepatocyte activities before and after cryopreservation. A single lot of human hepatocytes was functionally tested for transporter uptake after 2 hours, post-isolation and subsequent cryopreservation. Average accumulation of two substrates, (**A**) Na-taurocholate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

GIBCO® Human Hepatocytes, Induction Qualified

- Prequalified for CYP1A2, CYP2B6 and CYP3A induction
- ≥80% viability and ≥80% confluency (if plated cells)
- Minimum specific activities:
  - ≥10-fold induction of CYP1A2
  - ≥5-fold induction of CYP2B6
  - ≥3-fold induction of CYP3A4

Enzyme induction assay to qualify lots for use in experiments that monitor *in vitro* enzyme induction

Our induction-qualified hepatocytes have passed our test for specific activity and mRNA levels in response to prototypical inducers. We culture our cryopreserved human hepatocytes in 24-well collagen-coated plates and dose in triplicate with vehicle (0.1% DMSO), omeprazole (OMP), phenobarbital (PB) and rifampin (RIF) for 72 hours. Once monolayers are washed, they are incubated with substrates phenacetin, bupropion, and testosterone to determine CYP1A2, CYP2B6 and CYP3A activities, respectively (Table 2, Figure 7). Fold induction of specific activity is expressed as the ratio of induced activity to vehicle activity. mRNA content is also determined by TaqMan® qRT-PCR analysis after 48 hours treatment.

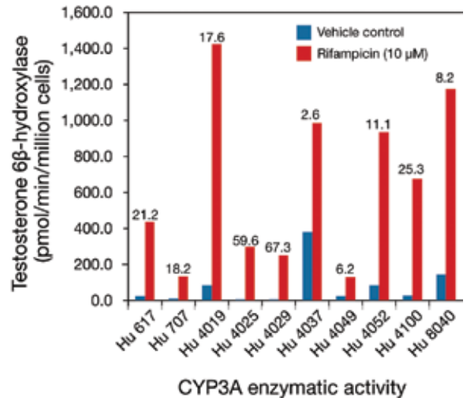


Figure 7. GIBCO® human cryopreserved hepatocytes tested for fold induction of CYP3A. GIBCO® human cryopreserved hepatocytes are tested for response to prototypical inducers to determine suitable applications. In this example, the fold induction of CYP3A (number shown above the bar line) is calculated,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

Table 2. Substrate probes for the assessment of P450 activity in GIBCO® Human Cryopreserved Plateable Hepatocytes, Induction Qualified.

Enzyme	Inducer	Inducer concentration	Substrate	Substrate concentration	Incubation time	Marker metabolite
CYP1A2	Omeprazole	50 µM	Phenacetin	100 µM	15 min	Acetaminophen
CYP2B6	Phenobarbital	1000 µM	Bupropion	500 µM	20 min	Hydroxybupropion
CYP3A	Rifampicin	10 µM	Testosterone	200 µM	14 min	6β-Hydroxytestosterone

GIBCO® Human Cryopreserved Hepatocytes, Plated Metabolism Qualified

- Useful for the assessment of intrinsic clearance (CL_{int}) in low-turnover compounds
- Prequalified according to CL_{int} of midazolam, tolbutamide, 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 P450 substrates midazolam, tolbutamide, and dextromethorphan, respectively (Figure 8). Using 48-well collagen-coated plates, hepatocytes are allowed to attach prior to incubations in duplicate in serum-free Williams Medium E, with reactions stopped with ice-cold acetonitrile at time points indicated in Table 3. Well contents are stored at –70°C prior to analysis. The disappearance of parent compound is monitored by LC/MS/MS and intrinsic clearance values determined by linear regression.

Table 3. Incubation conditions for CL_{int} in plated cryopreserved human hepatocytes.

Substrate	Concentration	Incubation time
Midazolam	0.50 µM	0, 1, 2, 4, 6, 8 hr
Tolbutamide	1.00 µM	0, 4, 6, 8, 18, 24 hr
Dextromethorphan	1.00 µM	0, 1, 2, 4, 6, 8 hr

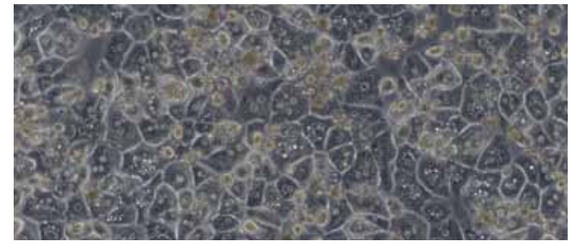


Figure 8. Cryopreserved human hepatocytes prequalified for plated metabolism (intrinsic clearance). CL_{int} (µL/1 x 10^6 cells/min) results were midazolam, 14.6; tolbutamide, 1.34; dextromethorphan, 7.20.

GIBCO® Human Cryopreserved Single Donor Hepatocytes, Metabolism Qualified

- Characterized for CYP1A2, CYP2B6, CYP2C8, CYP2C9, CYP2C19, CYP2D6, CYP3A, ECOD, 7-HCG, and 7-HCS activities (Table 4)
- High quality lots with batch sizes >500 vials
- Specialty lots available, including low CYP2D6 metabolizers

Our 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, Invitrogen offers one of the largest selections of single donor lots (Figure 9).

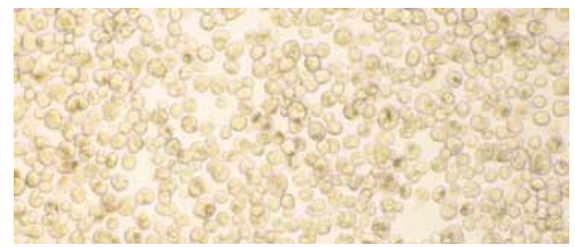


Figure 9. Human hepatocytes qualified for suspension metabolism applications.

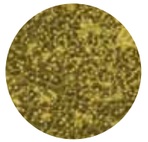


Table 4. Test conditions for determining the metabolic capacity of GIBCO® human cryopreserved suspension hepatocytes.

Enzyme	Substrate	Concentration	Incubation time	Marker metabolite
CYP1A2	Phenacetin	100 µM	15 min	Acetaminophen
CYP2B6	Bupropion	500 µM	20 min	Hydroxybupropion
CYP2C8	Paclitaxel	20 µM	45 min	6α-Hydroxypaclitaxel
CYP2C9	Diclofenac	25 µM	15 min	4′-Hydroxydiclofenac
CYP2C19	(S)-Mephenytoin	250 µM	30 min	4′-Hydroxymephenytoin
CYP2D6	Dextromethorphan	15 µM	15 min	Dextrorphan
CYP3A4	Testosterone	200 µM	14 min	6β-Hydroxytestosterone
Phase I and II	7-Ethoxycoumarin	100 µM	30 min	7-HCG, 7-HCS, 7-HC *

* 7-hydroxycoumarin glucuronide, 7-hydroxycoumarin sulfate, and 7-hydroxycoumarin

Table 5. Troubleshooting guide for plateable cryopreserved human hepatocytes.

Problem	Possible cause	Recommendation
Low cell viability, post-thaw	→ Improper thawing technique → Suboptimal thawing medium → Rough handling of hepatocytes during counting → Improper counting technique → Cells sat too long prior to counting or plating	→ Review Invitrogen's detailed thawing, plating, and counting protocols → Thaw cells <2 min at 37°C → Use CHRM® Medium during thawing to remove cryoprotectant → Mix slowly; use wide bore pipette tips → Ensure a homogenous cell mixture prior to counting → Count cells on 2 of the 4 grid lines → Do not let cells sit in trypan blue mixture for more than 1 min prior to loading → Plate cells immediately after counting
Unexpected cell yield	→ Improper thawing technique → Suboptimal thawing medium → Incorrect centrifugation speed → Rough handling of hepatocytes during counting → Improper counting technique	→ Review Invitrogen's detailed thawing, plating and counting protocols → Thaw cells <2 min at 37°C → Use CHRM® Medium during thawing to remove cryoprotectant → Centrifuge at 100 x *g* for 10 min at RT → Mix slowly; use wide bore pipette tips → Ensure a homogenous cell mixture prior to counting → Count cells on 2 of the 4 grid lines → Do not let cells sit in trypan blue mixture for more than 1 min prior to loading
Low attachment efficiency	→ Not enough time for cells to attach → Poor-quality substratum → Hepatocyte lot not characterized as plateable	→ Compare cultures to the pictures on Invitrogen's lot-specific characterization specification sheet → Wait before overlaying with Geltrex™ to see if attachment increases → Use GIBCO® Collagen I Coated Plates → Review Invitrogen's detailed thawing, plating and counting protocols (see above) → Check lot specifications to ensure it is qualified for plating

Table 5. Troubleshooting guide for plateable cryopreserved human hepatocytes, cont'd.

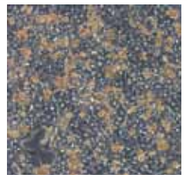
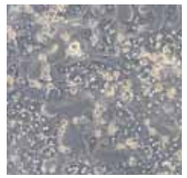
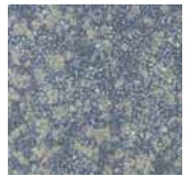
Problem	Possible cause	Recommendation
Suboptimal monolayer confluency	→ Seeding density too low → Insufficient dispersion of hepatocytes during plating → Insufficient plating volume used for well format → Low attachment efficiency (see above)	→ Check Invitrogen's lot-specific characterization specification sheet for appropriate seeding density → Observe cells under microscope for appropriate seeding prior to incubation → Disperse cells evenly by moving plate slowly in a figure-8 and back-and-forth pattern in incubator → Refer to Invitrogen literature or technical support for suggested plating volumes
Dirty monolayers (rounded-up cell clumps or debris on top of monolayer)	→ Seeding density too high → Insufficient dispersion of cells during plating → Improper plating volume used for well format	→ Check Invitrogen's lot-specific characterization specification sheet for appropriate seeding density → Observe cells under microscope for appropriate seeding prior to incubation → Disperse cells evenly by moving plate slowly in a figure-8 and back-and-forth pattern in incubator → Shake plate and wash cell monolayers prior to applying Geltrex™ overlay → Refer to Invitrogen literature or technical support for suggested plating volumes
Loss of membrane integrity or cuboidal cell shape	→ Hepatocyte lot not characterized as plateable → Suboptimal culture medium → Cells were cultured for too long	→ Check lot specifications to ensure it is qualified for plating → Use GIBCO® Williams Medium E with GIBCO® Plating and Incubation Supplement Packs → Refer to Invitrogen's plating protocol → In general, plateable cryopreserved human hepatocytes should not be cultured for more than 5 days
Suboptimal bile canalicular formation	→ Hepatocyte lot not transporter-qualified → Suboptimal culture medium → Not enough time for bile canaliculi to form	→ Check lot specifications to ensure it is transporter-qualified → Use GIBCO® Williams Medium E with GIBCO® Plating and Incubation Supplement Packs → Refer to Invitrogen's plating protocol → In general, a minimum of 4–5 days in culture is required for bile canalicular network formation
Unexpected induction results	→ Suboptimal monolayer confluency (see above) → Poor monolayer integrity (see above) → Inappropriate positive control → Incorrect concentration of positive control	→ Compare results to those reported on Invitrogen's lot-specific characterization specification sheet → Refer to Invitrogen's enzyme induction protocol → Check positive control to ensure suitability
Rounding up of cells, cellular debris, and/or holes in monolayer indicative of dying cells	→ Toxicity of test compound → Suboptimal culture medium → Hepatocyte lot not characterized as plateable → Cells were cultured for too long	→ Compare cell morphology of treated and nontreated cells → Refer to Invitrogen's plating protocol → Check lot specifications to ensure it is qualified for plating → In general, plateable cryopreserved human hepatocytes should not be cultured for more than 5 days

GIBCO® animal cryopreserved plateable and suspension hepatocytes

- → Major toxicology species available in plateable or suspension lots
- → Viabilities routinely >80%
- → Characterized for phase I and phase II enzyme activities

Our animal cryopreserved hepatocytes are prepared using the same careful isolation and cryopreserved techniques as our human lots. Invitrogen routinely isolates hepatocytes from mouse, rat, dog, rabbit, and nonhuman primate, with isolations from other species available upon request. 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.

Table 6. General guidelines used in the thawing and plating of cryopreserved hepatocytes.

	Human	Rat	Mouse	Dog	Monkey
Hepatocyte size	20 µm	30 µm	50 µm	20 µm	8–10 µm
Centrifuge conditions	100 *x g* 10 min	55 x *g* 3 min	55 x *g* 3 min	76 x *g* 6 min	76 x *g* 6 min
Plating density	0.7–0.9 x 10^6 total cells/mL	0.7–0.9 x 10^6 total cells/mL	0.3–0.5 x 10^6 total cells/mL	0.7–0.9 x 10^6 total cells/mL	0.9–1.1 x 10^6 total cells/mL
Culture after 72 hours					

Cell culture reagents

CHRM® Medium

Cryopreserved Hepatocyte Recovery Medium (CHRM®) is a proprietary formulation designed to enhance the recovery of viable hepatocytes while removing cryoprotectant after cell cryopreservation. When used appropriately, CHRM® has proven to result in healthier hepatocytes with consistently higher viability (Figure 10). CHRM® is easy to use: simply add one vial of thawed hepatocytes to the CHRM® conical tube, centrifuge briefly, remove medium, and gently resuspend cell pellet.

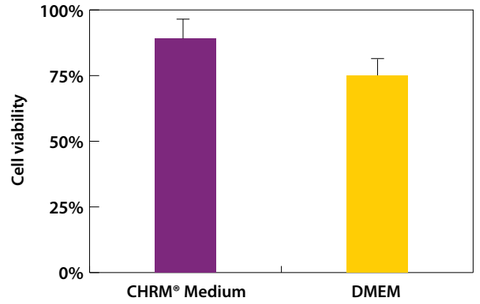


Figure 10. Comparison of CHRM® against DMEM in the ability to enhance hepatocyte viability post-cryopreservation. Data shown are averages of 126 studies of individual lots of human cryopreserved hepatocytes.

Williams Medium E

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

Hepatocyte Plating Supplement Pack

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

Hepatocyte Maintenance Supplement Pack

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

Geltrex™ 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 three-dimensional (3D) culture studies.

Collagen I, Rat Tail for Cell Culture

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.

Additional products for hepatic biology research

Invitrogen offers many products for the study of P450 metabolism, including:

- → P450 Baculosomes™ reagents—microsomes prepared from insect cells infected with a recombinant baculovirus expressing a specific human P450 enzyme and a rabbit NADPH-P450 reductase. Single isozymes that can be analyzed include CYP1A2, CYP2C9, CYP2C19, CYP2D6, CYP3A4, and CYP3A5. Baculosomes® reagents can also be used in conjunction with VIVID® substrates for fluorescence detection
- → LanthaScreen™ Nuclear Receptor Assays—including TR-FRET PXR Competitive Binding Assay and CAR Co-activator Assay
- → TaqMan® Drug Metabolism (DME) Assays—over 2,600 unique assays to detect polymorphisms in 220 genes that code for various drug metabolizing enzymes

Protocol for thawing and plating human and animal cryopreserved hepatocytes

Advance preparation: If using an overlay, refer to the Geltrex™ Reduced Growth Factor Basement Membrane Matrix (Invitrogen Cat. No. 12760-021) specification sheet for the lot's concentration and technical tips. Geltrex™ Matrix should be thawed on ice for 3–4 hours prior to application, or overnight at 4°C, and kept ice-cold.

Use universal safety precautions and appropriate biosafety cabinet when handing primary hepatocytes.

Thaw, spin, resuspend

1. Warm CHRM® Medium and Plating Medium (Williams Medium E supplemented with GIBCO® Hepatocyte Plating Supplement Pack, Serum-Containing) to 37°C for 15 min.
2. Thaw cryopreserved hepatocytes in 37°C water bath for ~2 min.
3. Wipe the vial with 70% alcohol in hood; pour or use wide-bore pipette tip to transfer hepatocytes into CHRM® Medium.
4. Centrifuge at room temperature:
   - → Human hepatocytes, 100 x *g* for 10 min
   - → Dog and nonhuman primate hepatocytes, 76 x *g* for 6 min
   - → Rat and mouse hepatocytes, 55 x *g* for 3 min
5. Aspirate carefully and add appropriate amount of Plating Medium (generally 1 mL per 1 x 10^6 cells).

Count, plate, and incubate

6. Determine cell viability and yield (for help, refer to hepatocyte counting instructions). If using cells in suspension, do not proceed with plating (Figure 11).
7. Dilute to seeding density with Plating Medium:
   - → Human hepatocytes—refer to Product Characterization Sheet (generally 0.7–0.9 x 10^6 total cells/mL)
   - → Dog and rat hepatocytes, 0.7–0.9 x 10^6 total cells/mL
   - → Mouse hepatocytes, 0.3–0.5 x 10^6 total cells/mL
   - → Nonhuman primate hepatocytes, 0.9–1.1 x 10^6 total cells/mL
8. Transfer hepatocytes to multi-well plate. Disperse, but do not swirl. For a 24-well plate:
   - → Human hepatocytes, 500 µL, density according to Product Characterization Sheet
   - → Dog and rat hepatocytes, 500 µL, 3.5–4.5 x 10^5 cells per well
   - → Mouse hepatocytes, 500 µL, 1.5–2.5 x 10^5 cells per well
   - → Nonhuman primate hepatocytes, 500 µL, 4.5–5.5 x 10^5 cells per well
9. Incubate plate at 37°C for 4–6 hr.
10. Agitate plates and aspirate medium.
11. If using an overlay, prepare 4°C Geltrex™ Matrix mixture by diluting ice-cold GIBCO® Geltrex™ Matrix with Williams Medium E containing GIBCO® Hepatocyte Maintenance Supplement Pack, Serum-Free, to a final concentration of 0.35 mg/mL.
12. Apply 4°C Geltrex™ overlay or 37°C Incubation Medium, and incubate plate overnight prior to use.

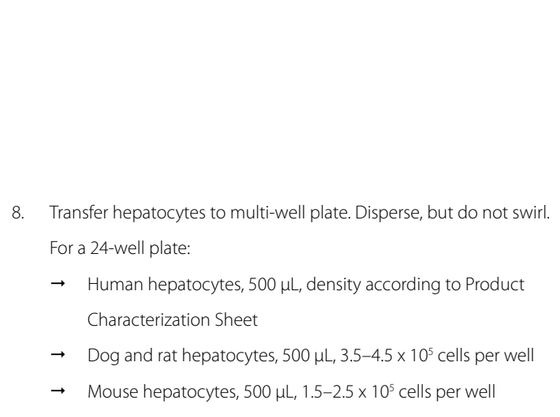


Figure 11. Setup for suspension vs. plated hepatocytes.

Protocol for counting primary hepatocytes using trypan blue exclusion analysis

Note: Use universal safety precautions and appropriate biosafety cabinet when handing primary hepatocytes. The procedure for thawing and removal of cryoprotectant from cryopreserved hepatocytes can be found in the plating protocol.

Prepare the cell counting vial

1. Mix 200 µL buffer (PBS or similar) with 50 µL 0.4% Trypan Blue stain in a 1.5 mL microcentrifuge tube. Transfer 50 µL of this diluted trypan blue solution into another 1.5 mL vial which will be used for counting the cells (this is the cell counting vial).
2. Before adding a portion of the hepatocyte suspension to the counting vial, gently invert the tube of hepatocytes several times to ensure a homogeneous solution. This step is crucial to obtaining an accurate cell count.
3. Using a wide-bore tip, pipette 50 µL of the hepatocyte suspension into the cell counting vial by drawing the solution into the pipette tip once and depressing the contents into the cell counting vial. Do not rinse out the tip. This results in a 2-fold dilution of the original cell suspension.
4. Gently flick/tap the counting vial to mix. Do not shake or agitate vigorously, as this will cause cell death and result in an inaccurate viability determination.

Prepare the hemocytometer

5. Wait one minute, gently flick/tap the counting vial again to mix, and then slowly pipette 10 µL of the mixture into the V-shaped groove on one side of the hemocytometer. Capillary action will pull the cell suspension into the hemocytometer.
6. Before counting, quickly scan the quadrants under the microscope to ensure the cells are evenly distributed. If the hemocytometer is loaded unevenly or contains a bubble, reload.

Count the hepatocytes

7. Immediately count the viable hepatocytes (clear or yellow color) and dead hepatocytes (blue color) in the four outer quadrants of the hemocytometer, including any cells on two of the outer quadrant lines (Figure 12). Do not delay in counting, as prolonged exposure to trypan blue can result in cell death.

Perform calculations

8. Determine total number of hepatocytes as well as percent of viable hepatocytes. Use additional supplemented Plating Medium or equivalent to dilute the hepatocyte mixture to the desired final cell concentration.
9. Percent viability = (live cells/total cells) x 100
10. Viable cell yield = average number of viable cells per quadrant (total live cells ÷ 4) x 10,000 x 2 x total volume (mL) of final cell suspension
11. Total cell density (cells/mL) = average number of viable + dead cells per quadrant x 10,000 x 2, where: 10,000 is the conversion factor for volume of one chamber quadrant (0.1 µL) and 2 is the cell dilution factor.

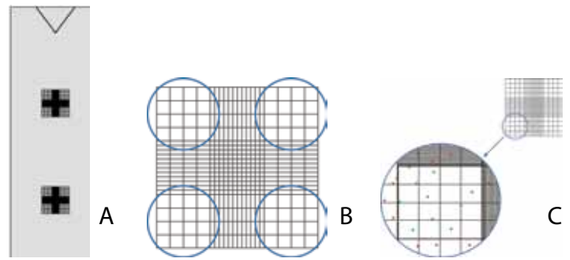
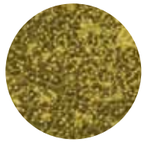


Figure 12. Counting cells on a hemocytometer. Count all four quadrants after loading the cell–trypan blue mixture onto one side of the hemocytometer (circled in B). When counting cells on the lines of the quadrants, two edges should be included in the cell count, and two edges should be excluded. In drawing C, for example, count the cells touching the lines on the top and left sides (green circles), but do not count the cells on the lines on the bottom and right sides (red circles).



Ordering information

Product	Quantity	Cat. No.
Human cryopreserved plateable hepatocytes **(Each lot's certificate of analysis has an exact cell count.)**		
Human Cryopreserved Plateable Hepatocytes, Transporter Qualified*	4–8 million viable cells	HMCPTS
Human Cryopreserved Plateable Hepatocytes, Transporter Qualified*	9–12 million viable cells	HMCPTL
Human Cryopreserved Plateable Hepatocytes, Induction Qualified	4–8 million viable cells	HMCPIS
Human Cryopreserved Plateable Hepatocytes, Induction Qualified	9–12 million viable cells	HMCPIL
Human Cryopreserved Plateable Hepatocytes, Metabolism Qualified	4–8 million viable cells	HMCPMS
Human Cryopreserved Plateable Hepatocytes, Metabolism Qualified	9–12 million viable cells	HMCPML
Human cryopreserved suspension hepatocytes		
Human Cryopreserved Suspension Hepatocytes, Transporter Qualified*	4–8 million viable cells	HMCSTS
Human Cryopreserved Suspension Hepatocytes, Transporter Qualified*	9–12 million viable cells	HMCSTL
Human Cryopreserved Suspension Hepatocytes, Metabolism Qualified, Male	4–8 million viable cells	HMCS1S
Human Cryopreserved Suspension Hepatocytes, Metabolism Qualified, Male	9–12 million viable cells	HMCS1L
Human Cryopreserved Suspension Hepatocytes, Metabolism Qualified, Female	4–8 million viable cells	HMCS2S
Human Cryopreserved Suspension Hepatocytes, Metabolism Qualified, Female	9–12 million viable cells	HMCS2L
Animal suspension and plateable cryopreserved hepatocytes **(Please inquire for minipig, guinea, and other custom species.)**		
Dog (Beagle) Cryopreserved Hepatocytes, Male	4–8 million viable cells	DGCS10
Dog (Beagle) Cryopreserved Hepatocytes, Female	4–8 million viable cells	DGCS20
Dog (Beagle) Cryopreserved Hepatocytes, Plateable Male	4–8 million viable cells	DGCP10
Dog (Beagle) Cryopreserved Hepatocytes, Plateable Female	4–8 million viable cells	DGCP20
Fish (Rainbow Trout) Cryopreserved Hepatocytes, Male	4–8 million viable cells	TRCS10
Fish (Rainbow Trout) Cryopreserved Hepatocytes, Female	4–8 million viable cells	TRCS20
Fish (Rainbow Trout) Cryopreserved Hepatocytes, Mixed Gender	4–8 million viable cells	TRCS30
Monkey (Cynomolgus) Cryopreserved Hepatocytes, Male	4–8 million viable cells	MKCS10
Monkey (Cynomolgus) Cryopreserved Hepatocytes, Female	4–8 million viable cells	MKCS20
Monkey (Cynomolgus) Cryopreserved Hepatocytes, Plateable Male	4–8 million viable cells	MKCP10
Monkey (Cynomolgus) Cryopreserved Hepatocytes, Plateable Female	4–8 million viable cells	MKCP20
Mouse (CD-1) Cryopreserved Hepatocytes, Male	4–8 million viable cells	MSCS10
Mouse (CD-1) Cryopreserved Hepatocytes, Female	4–8 million viable cells	MSCS20
Mouse (CD-1) Cryopreserved Hepatocytes, Plateable Male	4–8 million viable cells	MSCP10
Mouse (CD-1) Cryopreserved Hepatocytes, Plateable Female	4–8 million viable cells	MSCP20
Rabbit (New Zealand White) Cryopreserved Hepatocytes, Male	4–8 million viable cells	RBCS10
Rabbit (New Zealand White) Cryopreserved Hepatocytes, Female	4–8 million viable cells	RBCS20
Rabbit (New Zealand White) Cryopreserved Hepatocytes, Plateable Male	4–8 million viable cells	RBCP10
Rabbit (New Zealand White) Cryopreserved Hepatocytes, Plateable Female	4–8 million viable cells	RBCP20
Rat (Sprague-Dawley) Cryopreserved Hepatocytes, Male	4–8 million viable cells	RTCS10

* **Note**: GIBCO® Human Cryopreserved Hepatocytes, Transporter Qualified, are not prequalified for use with B-CLEAR® reagents and their purchase conveys no rights to practice the B-CLEAR® technology.

Ordering information, continued

Product	Quantity	Cat. No.
Rat (Sprague-Dawley) Cryopreserved Hepatocytes, Female	4–8 million viable cells	RTCS20
Rat (Sprague-Dawley) Cryopreserved Hepatocytes, Plateable Male	4–8 million viable cells	RTCP10
Rat (Sprague-Dawley) Cryopreserved Hepatocytes, Plateable Female	4–8 million viable cells	RTCP20
Media and reagents for hepatocyte culture		
Cryopreserved Hepatocytes Recovery Medium (CHRM®)	50 mL	CM7000
Williams' Medium E (1x, no phenol red)	500 mL	A1217601
Hepatocyte Plating Supplement Pack (Serum-containing)	1 kit for 500 mL medium	CM3000
Hepatocyte Maintenance Supplement Pack (Serum-free)	1 kit for 500 mL medium	CM4000
Cryopreserved Hepatocytes Plating Medium (CHPM)	50 mL	CM9000
Collagen I, Coated Plates, 6-well	1 x 5 plates	A1142801
Collagen I, Coated Plates, 24-well	1 x 5 plates	A1142802
Collagen I, Coated Plates, 96-well	1 x 5 plates	A1142803
Collagen I, Rat Tail for Cell Culture, 5 mg/mL	20 mL	A10483-01
Geltrex™ Reduced Growth Factor Basement Membrane Matrix	5 mL	12760-021

GIBCO® fresh hepatocytes

- Reliable supply from the industry's largest sourcing network
- Stringent release specifications: ≥80% viability and ≥80% confluency (plated cells)
- Ready for use—plated to your specifications, or provided in suspension

Primary human hepatocytes, under appropriate culture conditions, model metabolism, induction, and inhibition similar to the *in vivo* liver. Fresh human hepatocytes are used to test potential therapeutic compounds for their metabolic stability, metabolic profile, probability of hepatotoxicity, and ability to inhibit or induce metabolic enzymes and hepatic transporters. To achieve optimal *in vitro* results, primary hepatocytes must be carefully isolated to preserve their drug-metabolizing enzymes, transporters, and cellular activities. Our hepatocyte isolationists are experts in proper techniques to ensure optimal cell health. As a result, our fresh hepatocytes have high viabilities, excellent confluencies (Figure 13), and *in vivo*–like enzyme expression levels.

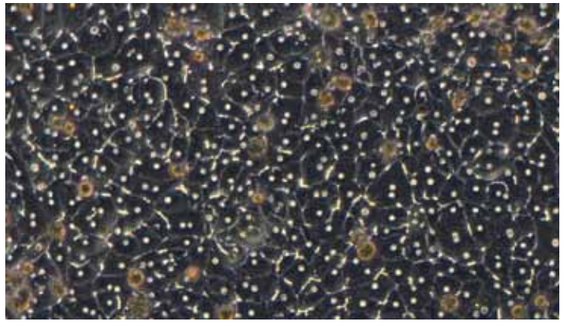


Figure 13. Human fresh hepatocytes shown post-plating on day 2. Good cellular morphology is indicated by the close cell–cell contacts, clearly defined nuclei, and cytosolic clarity.

GIBCO® animal fresh hepatocytes (North America only)

- Multiple isolations every week
- Published calendar to facilitate planning your experiments

We carry hepatocytes from the major toxicology species and accept many custom requests. Isolations are conducted weekly to facilitate your research. To view our animal isolation calendar, visit our website at www.invitrogen.com/hepatocytes. Species routinely available include:

- Rat (Sprague-Dawley)
- Mouse (CD-1)
- Dog (beagle)
- Nonhuman primate (cynomolgus)

Our formats are flexible—you can order hepatocytes in suspension, or let us do the plating for you. We plate our cells to maximum confluency (Tables 6 and 7), and offer an optional Geltrex™ Reduced Growth Factor Basement Membrane Matrix overlay to create a more *in vivo*–like environment.

- 6-well plates
- 12-well plates
- 24-well plates
- 48-well plates
- 96-well plates
- Suspension culture

GIBCO® human fresh hepatocytes

- Available in North America and Europe
- Weekly availability (typically)
- Rapid Alert™ emails keep you informed

At Invitrogen, we source liver tissue from a network of hospitals that perform surgical resections on living donors, as well as from deceased donors whose organs were rejected for transplant. Typically, we have access to one or more tissues per week. With such a large sourcing network, we can supply healthy human hepatocytes on a consistent basis, as well as keep you informed of tissues that meet your study's specific requirements. Refer to Table 8 to see where GIBCO® fresh hepatocytes are sold.

Rapid Alert™ notifications

Rapid Alert™ emails notify you right away about the impending availability of fresh primary human hepatocytes. When you receive a Rapid Alert™ email, just decide if you need hepatocytes, and contact us to place an order. Each Rapid Alert™ email contains:

- a summary of the donor's demographics (gender, age, race, BMI, and history of alcohol, drugs, or medications)
- plate and suspension formats available for order
- a shipping date for the hepatocytes
- a phone number and email to place your order or request additional information

To sign up for Rapid Alert™, access our online form at www.invitrogen.com/hepatocytes.

Table 6. Amount of GIBCO® fresh hepatocytes supplied per plate (millions of total cells).

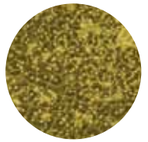
Format	Human	Rat	Mouse	Dog	Monkey
6-well plate	9.0	9.0	4.8	9.0	12.0
12-well plate	8.1	8.1	4.3	8.1	10.8
24-well plate	7.3	7.3	3.9	7.3	9.7
48-well plate	6.6	6.6	3.5	6.6	8.7
96-well plate	5.9	5.9	3.1	5.9	7.9

Table 7. Plating density of GIBCO® fresh hepatocytes plated onto GIBCO® collagen I–coated plates (total cells per well).

Format	Well volume	Human	Rat	Mouse	Dog	Monkey
6-well plate	2 mL	1.5×10^6	1.5×10^6	8.0×10^5	1.5×10^6	2.0×10^6
12-well plate	1 mL	6.7×10^5	6.7×10^5	3.6×10^5	6.7×10^5	9.0×10^5
24-well plate	500 µL	3.0×10^5	3.0×10^5	1.6×10^5	3.0×10^5	4.0×10^5
48-well plate	250 µL	1.4×10^5	1.4×10^5	7.3×10^4	1.4×10^5	1.8×10^5
96-well plate	125 µL	6.1×10^4	6.1×10^4	3.3×10^4	6.1×10^4	8.0×10^4
T75 flask	9 mL	12×10^6	12×10^6	N/A	12×10^6	12×10^6

Table 8. Regional availability of GIBCO® fresh human and animal hepatocytes.

Region	Human	Animal
Americas	Yes	Yes
Asia-Pacific	No	No
Europe	Yes	Coming soon
Middle East & Africa	Not yet	Not yet

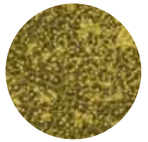


Ordering information

Product	Quantity	Cat. No.
Human and animal fresh suspension hepatocytes (There is a minimum order of 15 million cells.)		
Human Fresh Hepatocytes, Suspension	15 million cells	HMFS01
Dog (Beagle) Fresh Hepatocytes, Suspension	15 million cells	DGFS01
Monkey (Cynomolgus) Fresh Hepatocytes, Suspension	15 million cells	MKFS01
Monkey (Rhesus) Fresh Hepatocytes, Suspension	15 million cells	RHFS01
Mouse (CD-1) Fresh Hepatocytes, Suspension	15 million cells	MSFS01
Mouse (Balb/C) Fresh Hepatocytes, Suspension	15 million cells	BCFS01
Rat (Sprague-Dawley) Fresh Hepatocytes, Suspension	15 million cells	RTFS01
Human and animal fresh plated hepatocytes		
Human Fresh Hepatocytes	1 x 6-well plate	HMFN06
Human Fresh Hepatocytes	1 x 12-well plate	HMFN12
Human Fresh Hepatocytes	1 x 24-well plate	HMFN24
Human Fresh Hepatocytes	1 x 48-well plate	HMFN48
Human Fresh Hepatocytes	1 x 96-well plate	HMFN96
Human Fresh Hepatocytes, with Geltrex™ Overlay	1 x 6-well plate	HMFY06
Human Fresh Hepatocytes, with Geltrex™ Overlay	1 x 12-well plate	HMFY12
Human Fresh Hepatocytes, with Geltrex™ Overlay	1 x 24-well plate	HMFY24
Human Fresh Hepatocytes, with Geltrex™ Overlay	1 x 48-well plate	HMFY48
Human Fresh Hepatocytes, with Geltrex™ Overlay	1 x 96-well plate	HMFY96
Human Fresh Hepatocytes	1 x T75 flask	HMFN75
Dog (Beagle) Fresh Hepatocytes	1 x 6-well plate	DGFN06
Dog (Beagle) Fresh Hepatocytes	1 x 12-well plate	DGFN12
Dog (Beagle) Fresh Hepatocytes	1 x 24-well plate	DGFN24
Dog (Beagle) Fresh Hepatocytes	1 x 48-well plate	DGFN48
Dog (Beagle) Fresh Hepatocytes	1 x 96-well plate	DGFN96
Dog (Beagle) Fresh Hepatocytes, with Matrigel™ Overlay	1 x 6-well plate	DGFY06
Dog (Beagle) Fresh Hepatocytes, with Matrigel™ Overlay	1 x 12-well plate	DGFY12
Dog (Beagle) Fresh Hepatocytes, with Matrigel™ Overlay	1 x 24-well plate	DGFY24
Dog (Beagle) Fresh Hepatocytes, with Matrigel™ Overlay	1 x 48-well plate	DGFY48
Dog (Beagle) Fresh Hepatocytes, with Matrigel™ Overlay	1 x 96-well plate	DGFY96
Dog (Beagle) Fresh Hepatocytes	1 x T75 flask	DGFN75
Monkey (Cynomolgus) Fresh Hepatocytes	1 x 6-well plate	MKFN06
Monkey (Cynomolgus) Fresh Hepatocytes	1 x 12-well plate	MKFN12
Monkey (Cynomolgus) Fresh Hepatocytes	1 x 24-well plate	MKFN24
Monkey (Cynomolgus) Fresh Hepatocytes	1 x 48-well plate	MKFN48
Monkey (Cynomolgus) Fresh Hepatocytes	1 x 96-well plate	MKFN96

Ordering information, continued

Product	Quantity	Cat. No.
Monkey (Cynomolgus) Fresh Hepatocytes, with Geltrex™ Overlay	1 x 6-well plate	MKFY06
Monkey (Cynomolgus) Fresh Hepatocytes, with Geltrex™ Overlay	1 x 12-well plate	MKFY12
Monkey (Cynomolgus) Fresh Hepatocytes, with Geltrex™ Overlay	1 x 24-well plate	MKFY24
Monkey (Cynomolgus) Fresh Hepatocytes, with Geltrex™ Overlay	1 x 48-well plate	MKFY48
Monkey (Cynomolgus) Fresh Hepatocytes, with Geltrex™ Overlay	1 x 96-well plate	MKFY96
Monkey (Cynomolgus) Fresh Hepatocytes	1 x T75 flask	MKFN75
Mouse (CD-1) Fresh Hepatocytes	1 x 6-well plate	MSFN06
Mouse (CD-1) Fresh Hepatocytes	1 x 12-well plate	MSFN12
Mouse (CD-1) Fresh Hepatocytes	1 x 24-well plate	MSFN24
Mouse (CD-1) Fresh Hepatocytes	1 x 48-well plate	MSFN48
Mouse (CD-1) Fresh Hepatocytes	1 x 96-well plate	MSFN96
Mouse (CD-1) Fresh Hepatocytes, with Geltrex™ Overlay	1 x 6-well plate	MSFY06
Mouse (CD-1) Fresh Hepatocytes, with Geltrex™ Overlay	1 x 12-well plate	MSFY12
Mouse (CD-1) Fresh Hepatocytes, with Geltrex™ Overlay	1 x 24-well plate	MSFY24
Mouse (CD-1) Fresh Hepatocytes, with Geltrex™ Overlay	1 x 48-well plate	MSFY48
Mouse (CD-1) Fresh Hepatocytes, with Geltrex™ Overlay	1 x 96-well plate	MSFY96
Mouse (CD-1) Fresh Hepatocytes	1 x T75 flask	MSFN75
Rat (Sprague-Dawley) Fresh Hepatocytes	1 x 6-well plate	RTFN06
Rat (Sprague-Dawley) Fresh Hepatocytes	1 x 12-well plate	RTFN12
Rat (Sprague-Dawley) Fresh Hepatocytes	1 x 24-well plate	RTFN24
Rat (Sprague-Dawley) Fresh Hepatocytes	1 x 48-well plate	RTFN48
Rat (Sprague-Dawley) Fresh Hepatocytes	1 x 96-well plate	RTFN96
Rat (Sprague-Dawley) Fresh Hepatocytes, with Geltrex™ Overlay	1 x 6-well plate	RTFY06
Rat (Sprague-Dawley) Fresh Hepatocytes, with Geltrex™ Overlay	1 x 12-well plate	RTFY12
Rat (Sprague-Dawley) Fresh Hepatocytes, with Geltrex™ Overlay	1 x 24-well plate	RTFY24
Rat (Sprague-Dawley) Fresh Hepatocytes, with Geltrex™ Overlay	1 x 48-well plate	RTFY48
Rat (Sprague-Dawley) Fresh Hepatocytes, with Geltrex™ Overlay	1 x 96-well plate	RTFY96
Rat (Sprague-Dawley) Fresh Hepatocytes	1 x T75 flask	RTFN75



GIBCO® liver microsomes

- 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 cytosol

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 9) and are suitable for a variety of experiments:

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

Invitrogen provides liver subcellular fractions from a variety of toxicology species, including human. 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 select Phase II enzymes using FDA-recommended substrates (Tables 10, 11).

Table 9. Metabolic enzymes found in liver subcellular fractions.

Metabolic enzymes	Liver microsomes	Liver S9 fractions	Liver cytosol
Aldehyde oxidase		X	X
Cytochromes P450 (P450)	X	X	
Flavin monooxygenases (FMO)	X	X	
Glutathione transferase (GST)		X	
Monamine oxidase (MAO)		X	
Sulfurotransferases (SULT)		X	X
Uridine glucuronide transferase (UGT)	X	X	

Subcellular fractions available from these species:

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

Human and animal liver microsome thawing and incubation protocol

1. Prepare a 100X stock of the test article in solvent.
2. Thaw microsomes slowly on ice.
3. For a total of 198 µL, add the following:
   - 183 µL of 100 mM buffer
   - 10 µL of 20 mM NADPH (1 mM final concentration)
   - 5 µL microsomes (0.5 mg/mL final protein concentration)
4. Preincubate microsomes, buffer, and NADPH in water bath for 5 min.
5. Initiate the reactions with the addition of 2 µL 100X test article.
6. Incubate up to 60 min at 37°C with gentle agitation.
7. Terminate reactions by the addition of 200 µL organic solvent (i.e., ethyl acetate).
8. Add 25 µL internal standard to organic extract.
9. Vortex samples, and centrifuge at approximately 3,000 rpm for 5 min.
10. Transfer the organic layer to a clean plate and evaporate to dryness.
11. Reconstitute samples in mobile phase and analyze for metabolite formation by LC/MS/MS.

Table 10. 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	Dextrophan	3.2	0.13
CYP2E1	6-Hydroxychlorzoxazone	70	1.4
CYP3A4	6β-Hydroxytestosterone	19	4.0
CYP3A4	1′-Hydroxymidazolam	1.6	1.1

Table 11. Cytochrome P450 and Phase II enzymes tested in GIBCO® Human Liver Microsomes.

Enzyme monitored	Marker substrate	Incubation (min)	Protein concentration (mg/mL)	Metabolite
CYP1A1/2	Phenacetin	30	0.1	Acetaminophen
CYP2A6	Coumarin	5	0.025	7-Hydroxycoumarin
CYP2B6	Bupropion	20	0.25	Hydroxybupropion
CYP2C8	Paclitaxel	10	0.075	6α-Hydroxypaclitaxel
CYP2C9	Tolbutamide	20	0.1	Hydroxytolbutamide
CYP2C19	(S) Mephenytoin	30	0.1	4′-Hydroxymephenytoin
CYP2D6	Dextromethorphan	15	0.2	Dextrorphan
CYP2E1	Chlorzoxazone	20	0.1	6-Hydroxychlorzoxazone
CYP3A4/5	Midazolam	4	0.025	1′-Hydroxymidazolam
CYP3A4/5	Testosterone	7	0.05	6β-Hydroxytestosterone
CYP4A11	Lauric acid	15	0.2	12-Hydroxydecanoic acid
FMO	Methyl p-tolylsulfide	15	0.05	Methyl p-tolylsulfoxide
UGT	7-Hydroxycoumarin	30	0.2	7-Hydroxycoumarin glucuronide

Ordering information

Product	Quantity	Cat. No.
Human and animal liver microsomes, 20 mg/mL		
Human Microsomes, 50 Donors	0.5 mL	HMMCPL
Human Microsomes, Pooled Male Donors	0.5 mL	HMMCPM
Human Microsomes, Pooled Female Donors, 20 mg/mL	0.5 mL	HMMCPF
Human Microsomes, Single Donor	0.5 mL	HMMCSD
Human Microsomes, Pediatric Donors	0.5 mL	HMMCPE
Human Microsomes, Low Activity Donors	0.5 mL	HMMCLO
Human Microsomes, Obese Donors	0.5 mL	HMMCOB
Human Microsomes, Morbidly Obese Donors	0.5 mL	HMMCMO
Human Microsomes, Overweight Donors	0.5 mL	HMMCOW
Human Microsomes, Ideal Weight Donors	0.5 mL	HMMCIW
Human Microsomes, Over 75 Years Old Donors	0.5 mL	HMMC75
Human Microsomes, 18–39 Years Old Donors	0.5 mL	HMMC18
Human Microsomes, 40–60 Years Old Donors	0.5 mL	HMMC18
Human Microsomes, High 3A4 Activity Donors	0.5 mL	HMMC3A
Human Microsomes, High 2D6 Activity Donors	0.5 mL	HMMC2D
Dog (Beagle) Microsomes	0.5 mL	DGMCPL
Monkey (Cynomolgus) Microsomes	0.5 mL	MKMCPL
Monkey (Rhesus) Microsomes	0.5 mL	RHMCPL
Mouse (Balb/C) Microsomes	0.5 mL	BCMCPL
Mouse (CD-1) Microsomes	0.5 mL	MSMCPL
Rat (Sprague-Dawley) Microsomes	0.5 mL	RTMCPL
Mouse (CD-1) Control for Induced Microsomes, Corn Oil Vehicle	0.5 mL	MSMCVC
Mouse (CD-1) Microsomes, Induced with 3-Methylcholanthrene	0.5 mL	MSMCMC
Mouse (CD-1) Microsomes, Induced with Phenobarbital	0.5 mL	MSMCPB
Mouse (CD-1) Microsomes, Induced with Pregnenolone-16 Carbonitrile	0.5 mL	MSMCPC
Mouse (CD-1) Microsomes, Induced with Clorfibrate	0.5 mL	MSMCCL
Fish (Rainbow Trout) Microsomes, Male	0.5 mL	TRMC01
Fish (Rainbow Trout) Microsomes, Female	0.5 mL	TRMC02

Ordering information, continued

Product	Quantity	Cat. No.
Human and animal S9 fractions, 20 mg/mL		
Human S9 Fractions, Pooled	1.0 mL	HMS9PL
Human S9 Fractions, Single Donor	1.0 mL	HMS9SD
Dog (Beagle) S9 Fractions	1.0 mL	DGS9PL
Fish (Rainbow Trout) S9 Fractions	1.0 mL	TRS9PL
Monkey (Cynomolgus) S9 Fractions	1.0 mL	MKS9PL
Monkey (Rhesus) S9 Fractions	1.0 mL	RHS9PL
Mouse (Balb/C) S9 Fractions	1.0 mL	BCS9PL
Mouse (CD-1) S9 Fractions	1.0 mL	MSS9PL
Rat (Sprague-Dawley) S9 Fractions	1.0 mL	RTS9PL
Human and animal cytosol, 20 mg/mL		
Human Cytosol, Pooled	1.0 mL	HMCYPL
Human Cytosol, Single Donor	1.0 mL	HMCYSD
Dog (Beagle) Cytosol	1.0 mL	DGCYPL
Monkey (Cynomolgus) Cytosol	1.0 mL	MKCYPL
Monkey (Rhesus) Cytosol	1.0 mL	RHCYPL
Mouse (CD-1) Cytosol	1.0 mL	MSCYPL
Mouse (Balb/C) Cytosol	1.0 mL	BCCYPL
Rat (Sprague-Dawley) Cytosol	1.0 mL	RTCYPL

Ordering Information

For inquiries, or to place an order, please refer to the contact information listed on page 3. In North America, orders for fresh and cryopreserved hepatocytes are placed with our subsidiary, CellzDirect..

Shipping and storage of cryopreserved hepatocytes

Cryopreserved hepatocytes are shipped FOB Origin from Durham, NC (North America orders), from Inchinnan, UK (European Union orders), or potentially from an alternative distribution center (Asia-Pacific orders). Hepatocytes are shipped in the vapor phase of liquid nitrogen. Return instructions for the shipping dewar are included in each shipment.

Upon receipt, transfer cryopreserved hepatocytes to the laboratory cryostorage dewar or -135°C freezer. Transfer of the vials should occur in the shortest time possible (<1 min) to prevent warming that could result in decreased hepatocyte viability or cell yield. Store the vials of hepatocytes in the vapor phase of liquid nitrogen, below -130°C, until needed for use.

Shipping and storage of fresh hepatocytes

Fresh hepatocytes are shipped FOB Origin from Durham, NC (North America orders) or from Warrington, UK (European Union orders). Fresh suspension cells are sent in cold preservation solution via courier/commercial shippers and delivered as soon as possible. Plated hepatocytes are shipped via courier/commercial shippers one to two days post-plating. This is to ensure good cell health and stabilized P450 levels.

Upon receipt, fresh hepatocytes in suspension should be centrifuged at 4°C immediately, following our instructions. Plated hepatocytes should be quickly removed from the box, and the shipping medium replaced with fresh culture medium. The preservation medium used during shipment is meant to be kept cold; if the cells are warmed above 8°C, the shipping medium begins to have a toxic effect on the hepatocytes. We recommend that cells acclimate overnight prior to beginning your experiment.

Shipping and storage of microsomes and other liver subcellular fractions

Liver microsomes, S9 fractions, and cytosol are shipped FOB Origin from Durham, NC or Frederick, MD (North America orders), Inchinnan, UK (European Union orders), or potentially from an alternative distribution center (Asia-Pacific orders). Liver subcellular fractions are shipped on dry ice, unless the shipment needs to pass through customs, in which case they are shipped in a vapor shipper. Upon receipt, liver subcellular fractions should be immediately transferred to a -80°C freezer.

Learn more

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- → Cell counting of primary hepatocytes using trypan blue exclusion analysis
- → Metabolic stability using suspension hepatocytes
- → Metabolic stability using plated hepatocytes
- → Induction potential in plated hepatocytes

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- → Hepatocyte Expert Programs (HEP™)
- → Product Characterization Sheets with lot-specific data
- → Technical support and scientific consultation

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