1. How do I obtain human hepatocytes from Corning Life Sciences?
Register through Corning’s HepatoLink® online notification system at www.corning.com/lifesciences/hepatolink, or contact hepatocytes@corning.com.

2. What information is available regarding the human donors?
The donor information typically available includes: age, gender, race, social, over-the-counter and prescription drug use, significant medical history, and cause of death. In addition, serologies for a variety of pathogens are available.

3. Are Corning Gentest human hepatocyte products tested for biohazardous agents? Does Corning take any special safety precautions?
All human hepatocytes are derived from human tissue which is a potentially biohazardous material. Universal Precautions should be used when handling, which means the material should be handled as if it were capable of transmitting disease. Our cryopreserved hepatocytes are tested and found negative for Human Immunodeficiency Virus (HIV-1/HIV-2), Human T Cell Leukemia Virus (HTLV-1/HTLV-2), Hepatitis B Virus (HBV), Hepatitis C Virus (HCV), and Cytomegalovirus (CMV); however, no known test method can provide complete assurance that specimens of human origin will not transmit infectious disease. When handling or disposing, follow precautions described in CDC and FDA recommendations and OSHA bloodborne pathogen recommendations.

4. Can cryopreserved hepatocytes be thawed and refrozen?
We do not recommend refreezing hepatocytes. This process typically results in a significant loss of cell viability and yield.

5. When are hepatocytes to be used in drug discovery?
Hepatocytes can be used at any stage of drug discovery. They are the best in vitro model to determine toxicity, metabolic stability, enzyme induction, drug-drug interaction, and screening drug candidates.

6. How do hepatocytes compare to Human Liver Microsomes (HLMs) or Corning Supersomes™ enzymes?
Hepatocytes are whole cells, whereas microsomes and Corning Supersomes enzymes are subcellular fractions. Therefore, hepatocytes contain a complete phase I and phase II metabolism system while subcellular fractions do not. With hepatocytes, one can observe oxidative metabolites, glucuronide and sulfate conjugates directly. With HLMs, one can observe oxidative metabolism upon fortification with NADPH. To observe conjugation reactions, one needs to further fortify UDPGA for UGTs and other cofactors for other pathways. Generally, UGTs appear to be quantitatively less active in microsomes relative to hepatocytes. Corning Supersomes enzymes behave similarly to liver microsomes except the enzyme complement is more limited but highly defined.

7. How do hepatocytes compare to in vivo studies?
Hepatocytes are generally considered to be the closest thing to in vivo metabolism studies, although there is no standard for comparison.
8. Does Corning Life Sciences use hepatocytes for in vitro metabolism and/or induction studies?
Yes. Corning® Gentest™ inducible-qualified cryohepatocytes are ideal for studying induction of metabolic enzymes. Corning Gentest metabolism-qualified cryohepatocytes are ideal for the study of metabolism of drugs or drug candidate compounds.

9. What is 7-HFC (7-hydroxy 4-trifluoromethyl-coumarin) metabolic activity specific for – glucuronidation or sulfation?
In human hepatocytes, 7-HFC is primarily a substrate to measure UGT glucuronidation activity. There is some sulfation activity occurring, but it is minimal compared to glucuronidation in human hepatocytes.

10. Do I need to add cofactors or cytosol to hepatocytes?
No, hepatocytes contain all the necessary co-factors (e.g., NADPH and UDPGA) for both phase I and phase II drug metabolism.

11. What is the recommended density of (cryo)hepatocytes in a metabolism assay?
The recommended density of cryohepatocytes is $0.5 \times 10^6$ to $1.0 \times 10^6$ cells/mL.

12. Which solvents should be used with test compounds?
Dimethyl Sulfoxide (DMSO) is the preferred solvent and should be used in concentrations less than 0.2%. For induction experiments, the final DMSO concentration in the culture media should be less than 0.1%.

13. What is the density of human hepatocytes on plated flasks and multiwell plates?
Hepatocytes are seeded at $2.0 \times 10^5$ cells/cm². We recommend total protein quantitation of individual monolayers for the purpose of calibrating data of induction/enzyme studies.

14. Are Corning Gentest human hepatocytes always available from stock?
Cryopreserved human hepatocytes are in stock and can be purchased at any time.

15. How are enzyme activities in Corning Gentest hepatocytes characterized?
We have three categories of cryopreserved hepatocytes—metabolism-qualified, transporter-qualified, and inducible-qualified hepatocytes. The characterization assays are listed below:

**Human Cryohepatocytes, Metabolism-qualified:**
- Phenacetin O-deethylase (CYP1A2), bupropion hydroxylase (CYP2B6), amodiaquine N-demethylase (CYP2C8), diclofenac 4′-hydroxylase (CYP2C9), bufuralol 1′-hydroxylase (CYP2D6), testosterone 6β-hydroxylase (CYP3A), AZT-glucuronidation (UGT2B7), and 7-hydroxycoumarin-glucuronidation (non-selective UGT substrate).

**Human Cryohepatocytes, Transporter-qualified:**
- All the assays used for metabolism-qualified hepatocytes PLUS estrone-3-sulfate uptake (OATP), tetaethylammonium bromide uptake (OCT1), and taurocholate uptake (NTCP).

**Human Cryohepatocytes, Inducible-qualified:**
- All the assays used for metabolism-qualified PLUS induction of testosterone 6β-hydroxylase activity (CYP3A) by 20 µM rifampicin and induction of phenacetine O-deethylase activity (CYP1A2) by 20 µM β-naphtoflavone.

**Enzyme Activities in Cryopreserved Animal Hepatocytes:**
- **Rat and Mouse:** Testosterone 6β-hydroxylase, 7-hydroxycoumarin sulfation, and glucuronidation.
- **Dog:** The same assays as Rat and Mouse PLUS testosterone 16β-hydroxylase, phenacetin O-deethylase, bufuralol 1′-hydroxylase, and chlorzoxazone 6-hydroxylase.
- **Cynomologus Monkey:** The same assays as Rat and Mouse PLUS testosterone 16β-hydroxylase, testosterone 16β-hydroxylase, phenacetin O-deethylase, bufuralol 1′-hydroxylase, and p-nitrophenol hydroxylase.
For more specific information on claims, visit the Certificates page at www.corning.com/lifesciences.

**Warranty/Disclaimer:** Unless otherwise specified, all products are for research use only. Not intended for use in diagnostic or therapeutic procedures. Not for use in humans. Corning Life Sciences makes no claims regarding the performance of these products for clinical or diagnostic applications.

**Use of Genetically Modified Microorganisms (GMMO)**

Information for European Customers: Corning® immortalized hepatocytes, Corning Supersomes™ enzymes, Corning TransportoCells™ products are genetically modified microorganisms as described in Corning Life Sciences technical literature. As a condition of sale, use of these products must be in accordance with all applicable local guidelines on the contained use of genetically modified microorganisms, including the Directive 2009/41/EC of the European Parliament and of the Council.
At Corning, cells are in our culture. In our continuous efforts to improve efficiencies and develop new tools and technologies for life science researchers, we have scientists working in Corning R&D labs across the globe, doing what you do every day. From seeding starter cultures to expanding cells for assays, our technical experts understand your challenges and your increased need for more reliable cells and cellular material.

It is this expertise, plus a 160-year history of Corning innovation and manufacturing excellence, that puts us in a unique position to offer a beginning-to-end portfolio of high-quality, reliable cell culture consumables.