

Hepatocytes

Frequently Asked Questions

How do I obtain human hepatocytes from BD Biosciences?

Register through BD Biosciences' HepatoLink online notification system at www.bdbiosciences.com/admetox, or contact hepatocytes@BD.com.

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.

Are BD Gentest human hepatocyte products tested for biohazardous agents? Does BD 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. Due to the need to process and ship fresh hepatocytes quickly, only the donor serologies are available at the time of shipment. For cryopreserved hepatocytes, additional testing using FDA-approved methods 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.

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.

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.

How do hepatocytes compare to Human Liver Microsomes (HLMs) or BD Supersomes enzymes?

Hepatocytes are whole cells whereas microsomes and BD 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. BD Supersomes enzymes behave similarly to liver microsomes except that the enzyme complement is more limited but highly defined.

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.

Does BD Biosciences use hepatocytes for in vitro metabolism and/or induction studies?

Yes. BD Gentest™ inducible-qualified cryohepatocytes and fresh plated hepatocytes are ideal for studying induction of metabolic enzymes. BD Gentest metabolism-qualified cryohepatocytes are ideal for the study of metabolism of drugs or drug candidate compounds.

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.

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.

What is the recommended density of (cryo)hepatocytes in a metabolism assay?

The recommended density of cryohepatocytes is 0.5×10^6 - 1.0×10^6 cells/mL. Refer to the accompanying protocol for additional information.

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%.

What is the density of human hepatocytes on plated flasks and multiwell plates?

Hepatocytes are seeded at 2.0×10^5 cells/cm². We recommend total protein quantitation of individual monolayers for the purpose of calibrating data of induction/enzyme studies.

Are BD Gentest human hepatocytes always available from stock?

Cryopreserved human hepatocytes are in stock and can be purchased at any time. Fresh human hepatocytes are available upon receipt of fresh tissue. On average, we receive a fresh liver every week, so product is available on a consistent and convenient basis.

Do BD Gentest plated hepatocytes come with gel stabilizers to prevent the media from sloshing during shipment?

No. BD Gentest™ plated hepatocytes come with a special sealing gasket lid to minimize leakage of media during shipment, therefore the use of gel stabilizers is not necessary. Once delivered, the cardboard lid sealer can be cut open and the gasket lid can be replaced with the standard BD Falcon™ microplate lid enclosed with each shipment. BD Gentest plated hepatocytes in T-flasks do not require any special gaskets for shipment.

How are BD Gentest plated hepatocytes shipped to me? Do you use standard overnight delivery like Federal Express or Airborne?

Each shipment of BD Gentest plated hepatocytes are custom couriered to each account to ensure proper temperature control and rapid delivery. Most shipments are delivered using Sterling Couriers to ensure maximum viability upon receipt.

Will BD Biosciences ever ship hepatocytes on Saturdays or Sundays? Do I need to be prepared for unusual deliveries on weekends?

BD Gentest plated hepatocytes are grown in BD Hepatocyte Culture Media for maximum viability and longevity. It is generally not necessary to ship plated hepatocytes over weekends. Customers who register with BD Biosciences at www.bdbiosciences.com/admet or by emailing hepatocytes@gentest.com will be notified of any impending shipments. .

Is it expensive to custom courier plated hepatocytes to me? What sort of delivery charges can I expect?

All BD Gentest™ plated hepatocytes are shipped FOB Woburn, Massachusetts. Shipment charges vary with distance. To obtain a reasonable estimate for your location, contact hepatocytes@gentest.com and be sure to give your site location. Typical courier charges vary from approximately \$250-300/shipment in the US and approximately \$1,000/shipment outside the US.

How are enzyme activities in BD Gentest hepatocytes characterized?

We have three categories of cryopreserved hepatocytes-metabolism qualified, transporter qualified and platable inducible hepatocytes. The characterization assays are listed below:

Human CryoHepatocytes, Metabolism-Qualified:

Phenacetin O-deethylase (CYP1A2), bupropion hydroxylase (CYP2B6), amodioquine N-demethylase (CYP2C8), diclofenac 4'-hydroxylase (CYP2C9), bufuralol 1'-hydroxylase (CYP2D6), testosterone 6 β -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), tetraethylammonium bromide uptake (OCT1) and taurocholate uptake (NTCP).

Human CryoHepatocytes, Inducible-Qualified:

All the assays used for metabolism-qualified PLUS induction of testosterone 6 β -hydroxylase activity (CYP3A) by 20 μ M rifampicin and induction of phenacetin O-deethylase activity (CYP1A2) by 20 μ M β -naphthoflavone.

Enzyme Activities in Fresh Plated Human Hepatocytes:

Testosterone; (measuring CYP3A4 activity):

7-Methoxy-4-(trifluoromethyl)-coumarin (7-MFC); measuring multiple CYP isoform activities

7-Hydroxy-4-(trifluoromethyl)-coumarin (7-HFC); measuring multiple UGT glucuronidation isoforms

Enzyme Activities in Cryopreserved Animal CryoHepatocytes:

Rat and Mouse:

Testosterone 6 β -hydroxylase, 7-hydroxycoumarin sulfation and glucuronidation.

Dog:

The same assays as Rat and Mouse PLUS testosterone 16 α -hydroxylase, phenacetin O-deethylase, bufuralol 1'-hydroxylase and chlorzoxazone 6-hydroxylase.

Cynomologus Monkey:

The same assays as Rat and Mouse PLUS testosterone 16 α -hydroxylase, testosterone 16 β -hydroxylase, phenacetin O-deethylase, bufuralol 1'-hydroxylase and p-nitrophenol hydroxylase.



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