BIOIVT

ELEVATING SCIENCE

Researchers' Guide to Products for ADME-Tox Testing

Life Sciences/Biology

c&en BRANDLAB

TABLE OF CONTENTS

Photo credit: BioIVT

1 Introduction

1.1. What are ADME Products?

ADME products refer to tools and technologies used to study drug absorption, distribution, metabolism, and excretion (ADME) within the body. These products are crucial for *in vitro* models to evaluate pharmacokinetics, metabolic activity, drug-drug interaction potential, and toxicity risks associated with pharmaceuticals. ADME models enable researchers to predict a drug's behavior in the human body, optimize dosing, and assess safety and efficacy before clinical trials.

BioIVT offers a comprehensive portfolio of ADME products, including:

- Hepatocytes
- Kupffer cells
- Hepatic multicellular systems
- Subcellular fractions
- Recombinant enzymes

1.2. About BioIVT: Industry-Leading ADME Products for Research

BioIVT is the global leader in products for *in vitro* ADME research. The company pioneered the cell isolation and cryopreservation techniques that are now industry norms and continues to set the standard for high-quality and ethically sourced hepatocytes, subcellular fractions, and recombinant enzymes used in ADME research.

As part of its comprehensive biospecimen portfolio, BioIVT offers a range of products made with proprietary technologies, including pooled hepatocytes and long-term cultures. With its extensive inventory, BioIVT assists researchers in completing a study or course of studies with a single lot or set of lots, allowing researchers to produce reliable and reproducible data throughout their studies. The company combines technical expertise with exceptional customer service to provide the research community unmatched access to the products required for ADME and DMPK research.

View BioIVT's Comprehensive ADME Product Portfolio.

1.3. Why Partner with BioIVT for ADME Products?

For over 25 years, BioIVT has established its reputation as a reliable partner for ADME products. BioIVT scientists have isolated hepatocytes from several thousand human livers, and they continue to add hundreds of new lots each year. The company also maintains ready-to-ship inventory of standard products and can provide custom collections on request.

Hepatocytes are isolated from whole livers and are cryopreserved on the day of isolation. BioIVT's advanced techniques enable a high number of vials to be obtained from each liver, contributing to large lot sizes that demonstrate high viability and consistent reproducibility. The rigorous quality control process includes extensive evaluation of each lot. Characterization data are provided on easy-to-read certificates of analysis (CoAs).

Characterization data include:

- Viability
- Yield
- Monolayer confluency
- CYP induction
- Genotype
- Cytochrome P450 (CYP) and UDP-glucuronosyltransferases (UGT) metabolism
- NAS score

BioIVT has invested in logistics infrastructure to ensure ADME products can be delivered efficiently and quickly to researchers' laboratories. The company stages products at its US and European distribution centers to reduce shipping time and works with distributors across Asia. This approach ensures that products are close to final delivery locations and have already cleared country customs when clients request them.

As part of its commitment to rapid product delivery, BioIVT invested in cryogenic storage and can store more than one million vials. The company also maintains a fleet of dewars, which allows it to package and ship cells the same day orders are received.

"BioIVT is uniquely positioned to help our customers accelerate their preclinical drug development programs. Our technical experts assist scientists with selecting the right ADME test systems for their needs, help them find hepatocyte lots with the characteristics that meet their specifications, and routinely advise on culture techniques and applications.

Additionally, our research services group helps our clients design and implement ADME strategies to help them achieve their drug R&D goals, whether that's lead selection and optimization, advancing an asset to proof of concept for out-licensing, or taking a drug through investigational new drug (IND) submission and clinical development."

Dr. Chris Black, BioIVT senior vice president ADME-Tox

For information about ordering BioIVT's products, please see Appendix I.

1.4. Quality Assurance and Ethical Sourcing

BioIVT collects biospecimens under applicable laws, regulations, and ordinances. BioIVT's Regulatory and Quality office manages biospecimen collection protocols and ensures that the materials are ethically procured and that processes adhere to data privacy regulations such as GDPR and CCPA/CPRA. BioIVT complies with the Human Tissue Authority (HTA) regulations (UK) research and human applications.

Learn more about BioIVT's certifications and accreditations, informed consent processes, donor information practices, and compliance with other applicable regulations at BioIVT's website.

BioIVT receives human tissues from Research Tissue Organizations (RTOs) who work directly with Organ Procurement Organizations (OPOs) and facilitate the screening and allocation of organs post-mortem that do not meet transplant criteria and have informed consent or authorization for research use (which includes genetic research) and education and does not preclude commercial or international use.

BioIVT isolates hepatocytes and other cells and subcellular fractions per its protocols. These materials are then cryopreserved and extensively characterized after thawing.

Learn more about BioIVT's processes to isolate and characterize hepatocytes.

1.5. Test Systems and Applications

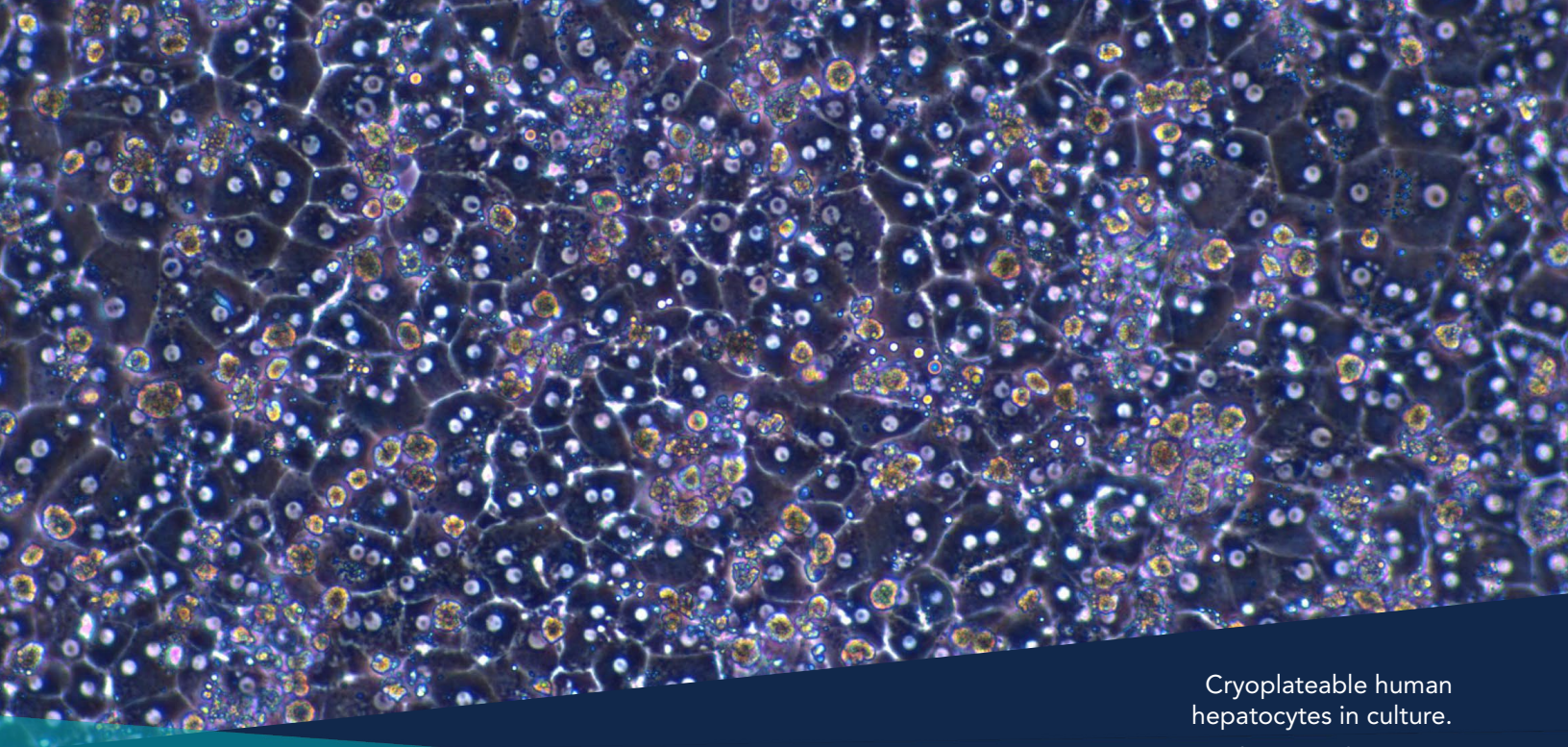
The ideal test system for an *in vitro* ADME study depends on the application and the research goals. For example, programs to develop the definitive data package for an IND submission may require more rigor and different design than is adequate for lead selection and optimization.

Table 1 summarizes major categories of test systems and ranks them for their suitability in specific applications.

Table 1: Major ADME Test Systems

Application	Cryopreserved hepatocytes	Kupffer cells	Pooled S9/ cytosol/ homogenate	Pooled microsomes	Individual microsomes/ S9/cytosol	Recombinant enzymes (rCYPs)	Lysosomes/ tritosomes
Species differences	+++	–	++	++	+	++	+
Metabolic stability	+++	–	++	++	+	+	+++
In vitro Toxicity	+++	+	++	+	–	–	–
Enzyme induction	+++	–	–	–	–	–	–
P450 inhibition	+++	–	+	+++	+	++	–
Reaction phenotyping	+++	–	++	+++	+	++	–
Integrated metabolism	+++	–	++	+	–	–	+
Uptake transporter assays	+++	–	–	–	–	–	–
Efflux transporter assays	+++	–	–	–	–	–	–
Genetic polymorphisms	+++	–	–	–	+++	++	–
3D hepatic models	+++	++	–	–	–	–	–
Low-turnover compounds	+++	–	–	–	–	–	–
CGT efficacy	+++	–	–	–	–	–	–
CGT off-target effects/toxicity	+++	+	–	–	–	–	–

+++ = Preferred /best **++** = Good **+** = Acceptable **–** = Not recommended



Cryoplateable human hepatocytes in culture.

Photo credit: BioIVT

2 Hepatocytes

2.1. Introduction

Primary hepatocytes are often referred to as the gold standard for *in vitro* ADME-Tox assays. They make up approximately 80% of the liver by mass and produce abundant amounts of most drug-metabolizing enzymes.

With good cell culture techniques, hepatocyte-specific cellular function can be maintained *in vitro*. This makes hepatocytes an ideal model for recapitulating liver function by means of xenobiotic metabolism, hepatotoxicity, cellular and gene therapy, and host-pathogen interactions. In drug development, hepatocytes are best suited for metabolism, clearance, transport, hepatotoxicity, and drug-drug interaction assays, making them a cost-effective alternative to *in vivo* testing.

BioIVT offers human hepatocytes in cryoplateable and cryosuspension formats as standard single-donor and pooled-donor lots. Custom-pooled lots are also available.

Table 2 summarizes BioIVT's major cryopreserved hepatocyte categories and products and ranks them for suitability in specific applications.

Table 2: Human Cryopreserved Hepatocyte Products

	Individual-donor cryosuspension hepatocytes	Individual-donor cryoplateable hepatocytes	Pooled cryosuspension hepatocytes	Pooled cryoplateable hepatocytes	HEPATOPAC/ HEPATOMUNE*	TRANSPORTER CERTIFIED®**	SPHEROID CERTIFIED™**
Species differences	+	++	+++	+++	++	++	++
Metabolic stability	+	+	+++	+++	++	++	++
In vitro Toxicity	–	+++	–	+++	+++	+++	+++
Enzyme induction	–	+++	–	++	++	+++	+++
P450 inhibition	+	+	++	++	+	+	+
Reaction phenotyping	+	+	++	++	++	+	+
Integrated metabolism	+++	++	+++	++	++	++	++
Uptake transporter assays	+	+	++	++	++	+++	+
Efflux transporter assays	–	++	–	++	++	+++	++
Genetic polymorphisms	+++	+++	+	+	+	–	–
3D hepatic models	–	++	–	+++	–	++	+++
Low-turnover compounds	–	++	+	++	+++	++	+++
CGT efficacy	–	+++	–	++	++	+++	+++
CGT off-target effects/toxicity	–	+++	–	++	+++	+++	+++

+++ = Preferred /best **++** = Good **+** = Acceptable **–** = Not recommended

*HEPATOPAC® and HEPATOMUNE® cultures are optimized for long-term exposure studies.
**These products are individual-donor cryoplateable hepatocytes that have been evaluated for specific applications.

2.2. Tips on Use: Storage, Thawing, Plating

Implementing studies using cryopreserved primary hepatocytes is straightforward when handled appropriately.

Storing
Cryopreserved hepatocytes must be stored below –150 °C in a temperature-controlled cryogenic freezer. If the vials need to be transported, they can be placed under dry ice for less than 30 minutes, but this time should be minimized. Vials should be stored in the vapor phase of LN2 storage.

Thawing
When thawing a vial of cryopreserved hepatocytes, do not overthaw the vial. A loose ice pellet should remain. The vial contents can then be decanted into a conical tube.

BioIVT offers cryopreserved hepatocytes that can be plated. These cryoplateable hepatocytes do not require a Percoll purification or washing step to achieve high viability. The cells must be thawed promptly, and the contents must be poured into a prewarmed medium (37 °C) to complete thawing. BioIVT recommends approximately 1 mL of the appropriate medium for every million cells per vial thawed.

Quality tip: Overthawing decreases cell viability. Diluting the cryopreservation solution as soon as possible after thawing will minimize damage to the cells.

Watch this guide to thawing and reconstitution of cryopreserved hepatocytes in suspension.

Watch this guide for thawing and plating cryoplateable hepatocytes.

Counting
An accurate cell count is essential for calculating the concentration in a cell suspension culture. BioIVT recommends the trypan blue exclusion counting method using a Neubauer hemocytometer to determine cell viability and concentration.

Click here to watch a video guide to cell counting using the trypan blue exclusion method.

Plating
Proper plating techniques ensure cells are distributed evenly and will have consistent well-to-well confluency. Refer to BioIVT's hepatocyte Instructions For Use guides for recommended seeding densities, culture media, and plating tips.

Note: *BioIVT's CryostaX® cells have different thawing methods. Please refer to page 11.*

Watch this guide for thawing and plating cryoplateable hepatocytes.

2.3. Human Cryoplateable Hepatocytes

Human cryoplateable hepatocytes from BioIVT are highly characterized for post-thaw viability and yield, metabolic activity rates, monolayer confluency, CYP induction, and for use in specialized applications. **Table 3** summarizes the major human cryoplateable hepatocyte products BioIVT offers as standard catalog products.

Table 3: Human Cryoplateable Hepatocytes

Description	Gender	Donors
Human cryoplateable single-donor and LIVERPOOL® hepatocytes	Female, male, mixed	1, 5, 10
Human CryostaX® cryoplateable single-donor and pooled hepatocytes	Female, male, mixed	1, 5, 10
Human cryoplateable TRANSPORTER CERTIFIED® hepatocytes	Female, male	1
Human cryoplateable, SPHEROID CERTIFIED hepatocytes	Female, male	1

For a full list of available human cryoplateable hepatocytes, see Appendix II.

2.3.1. Single-Donor Human Cryoplateable Hepatocytes

Human cryoplateable hepatocytes are primary hepatocytes that demonstrate attachments for up to 5 days in culture. These hepatocytes are isolated from intact human livers that are cryopreserved on the day of isolation. They undergo extensive evaluation to assess metabolic activity and induction levels to ensure consistency and reliability.

Thawing and Plating

Use the recommended media and antibiotic mix for thawing and plating human cryoplateable hepatocytes. For more information, read the human cryoplateable hepatocytes thawing and plating guide.

Learn more about single-donor human cryoplateable hepatocytes.

2.3.2. LIVERPOOL® Cryoplateable Hepatocytes

LIVERPOOL® cryoplateable hepatocytes are prepared from individual human cryoplateable hepatocytes and are offered in standard 5-donor and 10-donor formats. These large multidonor lots are formulated to have specific activity levels. LIVERPOOL cryoplateable hepatocytes can be cultured for up to 5 days.

Thawing and Plating
Use the recommended media and antibiotic mix for thawing and plating human cryoplateable hepatocytes. For more information, read the LIVERPOOL cryoplateable hepatocytes thawing and plating guide.

Learn more about Human LIVERPOOL cryoplateable hepatocytes.

2.3.3. CryostaX® Cryoplateable Hepatocytes (Individual and Pooled Donor)

CryostaX® hepatocytes are cryopreserved using CryostaX "single-freeze" pellet technology. Pooled CryostaX hepatocytes undergo only one freeze-thaw cycle rather than the two cycles necessary for conventional pooling. This process minimizes cryoinjury and allows for simplified, less-variable hepatocyte thaws and customization of hepatocyte pools. CryostaX hepatocytes are available in individual and pooled 5- or 10-donor formats.

Thawing and Plating
Use the recommended media for thawing and culturing hepatocytes. For more information, read the CryostaX cryoplateable hepatocytes thawing and plating guide.

Learn more about CryostaX human individual hepatocytes.

Learn more about CryostaX human pooled hepatocytes.

2.3.4. TRANSPORTER CERTIFIED® Hepatocytes

TRANSPORTER CERTIFIED® hepatocytes are cryoplateable human hepatocytes validated to have *in vivo*–relevant transporter function under culture conditions that BioIVT has defined. In sandwich culture, TRANSPORTER CERTIFIED hepatocytes simulate the bile pockets in bile canaliculi. They demonstrate the physiological transporter function, metabolic competence, and regulatory pathways that enable the expression of uptake and efflux transporters.

TRANSPORTER CERTIFIED® hepatocytes are ideal for whole-cell models, including hepatobiliary disposition using B-CLEAR technology (i.e., B-CLEAR studies), cholestatic hepatotoxicity using the C-DILI assay, enzyme induction and hepatoxicity assays, and other models that incorporate hepatobiliary efflux.

Learn more about TRANSPORTER CERTIFIED hepatocytes.

2.3.5. SPHEROID CERTIFIED™ Hepatocytes

Human SPHEROID CERTIFIED™ hepatocytes are cryoplateable hepatocytes that have been shown to have long-term viability in 3D cultures. They are ideal for hepatic spheroid models that are used to assess drug metabolism, toxicity, inflammation, and other pharmacokinetic effects. These models can also incorporate other cell types to develop systems with greater physiological relevance.

Thawing and Plating
Use the recommended media and antibiotic mix to thaw, plate, and maintain spheroid-forming hepatocytes. For more information, read the SPHEROID CERTIFIED hepatocytes thawing and plating guide.

Learn more about SPHEROID CERTIFIED hepatocytes.

2.4. Human Cryosuspension Hepatocytes

Human cryosuspension hepatocytes from BioIVT are highly characterized and evaluated to assess metabolic activity levels, ensuring consistency and reliability. Cryosuspension hepatocytes are an ideal test system for metabolic stability assays and other studies in which long-term cultures are not required. **Table 4** summarizes the BioIVT's human cryosuspension hepatocyte standard catalog products.

Table 4: Human Cryosuspension Hepatocytes

Description	Gender	Donors
Human cryosuspension single-donor and LIVERPOOL® hepatocyte	Female, male, mixed	1, 5, 10, 20, 50, 200
Human CryostaX® cryosuspension single-donor and pooled hepatocytes	Female, male, mixed	1, 10, 20

For a full list of available human cryosuspension hepatocytes, see Appendix III.

2.4.1. Single-Donor Cryosuspension Hepatocytes

Human cryosuspension hepatocytes are cryopreserved human hepatocytes that demonstrate high functionality in suspension cultures.

Thawing and Culturing
Use recommended media for thawing, cell counting, and culture. For more information, read the single-donor cryosuspension hepatocytes thawing and culture guide.

Learn more about single-donor human cryosuspension hepatocytes.

2.4.2. LIVERPOOL® Cryosuspension Hepatocytes

LIVERPOOL® cryosuspension hepatocytes are pooled human hepatocytes formulated to have specific activity levels and remain viable in culture for 4 - 6 hours. All LIVERPOOL lots meet strict quality-control specifications and are available in 5-, 10-, 20-, 50-, and 200-donor formats.

Thawing and Culturing
Use the recommended media for thawing, cell counting, and culture. For more information, read the LIVERPOOL cryosuspension hepatocytes thawing and culture guide.

Learn more about LIVERPOOL human cryosuspension hepatocytes.

2.4.3. CryostaX® Cryosuspension Hepatocytes (Individual and Pooled Donor)

CryostaX® hepatocytes are cryopreserved primary hepatocytes using CryostaX single-freeze pellet technology. This patented single-freeze preparation minimizes cryoinjury, promoting high viability, cell yield, and enzymatic activity for long-term consistent test results. CryostaX pooled cryosuspension hepatocytes are offered in standard 10- and 20-donor formats. These hepatocytes are extensively characterized and can be custom-pooled on demand.

Thawing and Culturing
Use the CryostaX Thawing Hepatocyte Kit to thaw CryostaX hepatocytes. The kit is essential to maximizing viability and cell yield for each CryostaX hepatocyte lot. It includes cell culture media optimized for thawing cryopreserved hepatocytes and CryostaX counting solution for determining cell yield and viability counts. Read the CryostaX thawing guide.

Learn more about CryostaX individual human cryosuspension hepatocytes.

Learn more about CryostaX pooled human cryosuspension hepatocytes.

2.5. Fresh Hepatocytes

Fresh human hepatocytes are isolated from whole livers and prepared in suspension or plate formats, including collagen and Matrigel overlay for sandwich cultures. Hepatocyte suspensions are typically shipped the same day the livers are received, while freshly plated hepatocyte products, including sandwich cultures, are shipped the day after the cells have been attached.

Fresh human hepatocytes are used in many *in vitro* ADME models, including studies of hepatic metabolism, CYP induction, hepatotoxicity, and drug transporter activity.

Instructions for Use
Use the recommended media for cell counting and plating. For more information, read the instructions for using fresh suspension hepatocytes and instructions for using fresh plated hepatocytes.

Learn more about fresh human hepatocytes.

2.6. Animal Hepatocytes

Metabolic differences among species can significantly affect the outcomes of ADME studies. If these differences are understood, an animal model that will represent human metabolism can be selected. **Table 5** summarizes key metabolic enzymes that differ among commonly used model species.

Table 5. Overview of Metabolic Traits of Animal Hepatocytes

Animal	Prominent metabolic traits
Human	Single functional aldehyde oxidase (AO); no carboxylesterase in blood; dopamine oxidized by monoamine oxidase (MAO)-B; flavin monooxygenase (FMO) 3 is the dominant hepatic FMO; quaternary N-glucuronide formation
Mouse	Four functional AO genes; carboxylesterase activity in blood; dopamine oxidized by MAO-A; FMO1 is the dominant hepatic FMO; no quaternary N-glucuronide formation
Rat	Four functional AO genes; carboxylesterase activity in blood; dopamine oxidized by MAO-A; FMO1 is the dominant hepatic FMO; no quaternary N-glucuronide formation
Dog	No AO; no carboxylesterase in blood and intestine; FMO1 and FMO3 are the dominant hepatic FMOs; quaternary N-glucuronide formation
Minipig	One functional AO; no carboxylesterase in blood; FMO1 is the dominant hepatic FMO
Nonhuman primate (NHP)	Two functional AOs; no carboxylesterase in blood; FMO3 is the dominant hepatic FMO; quaternary glucuronide formation

Selecting a species that accurately represents human metabolism will enhance the predictive value of nonclinical ADME studies. However, there are many considerations when choosing a nonhuman animal. Critical steps include:

Analyze test article structure: Analyze the structure of the test article and predict its potential metabolism.

Select appropriate test systems: Use test systems such as hepatocytes or subcellular fractions to determine the metabolic clearance and metabolite profiles across species.

Metabolite profiles comparison: Compare metabolite profiles across different species.

Consider other aspects: Evaluate other aspects of optimal absorption, distribution, metabolism, excretion, pharmacokinetics (ADME/PK), and pharmacology.

BioIVT offers a variety of hepatocytes from clinically relevant species in cryopreserved and fresh formats. For a complete list of available animal hepatocytes, see Appendix IV. BioIVT also offers custom animal hepatocytes to match experimental needs.

Photo credit: BioIVT

3 Human Kupffer Cells

3. Introduction

Researchers use BioIVT's Kupffer cells in a variety of *in vitro* screening assays, toxicity studies, and disease models to predict inflammation-induced adverse drug reactions. The cells can be used in plated monocultures, cocultures, liver microtissues, or 3D configurations.

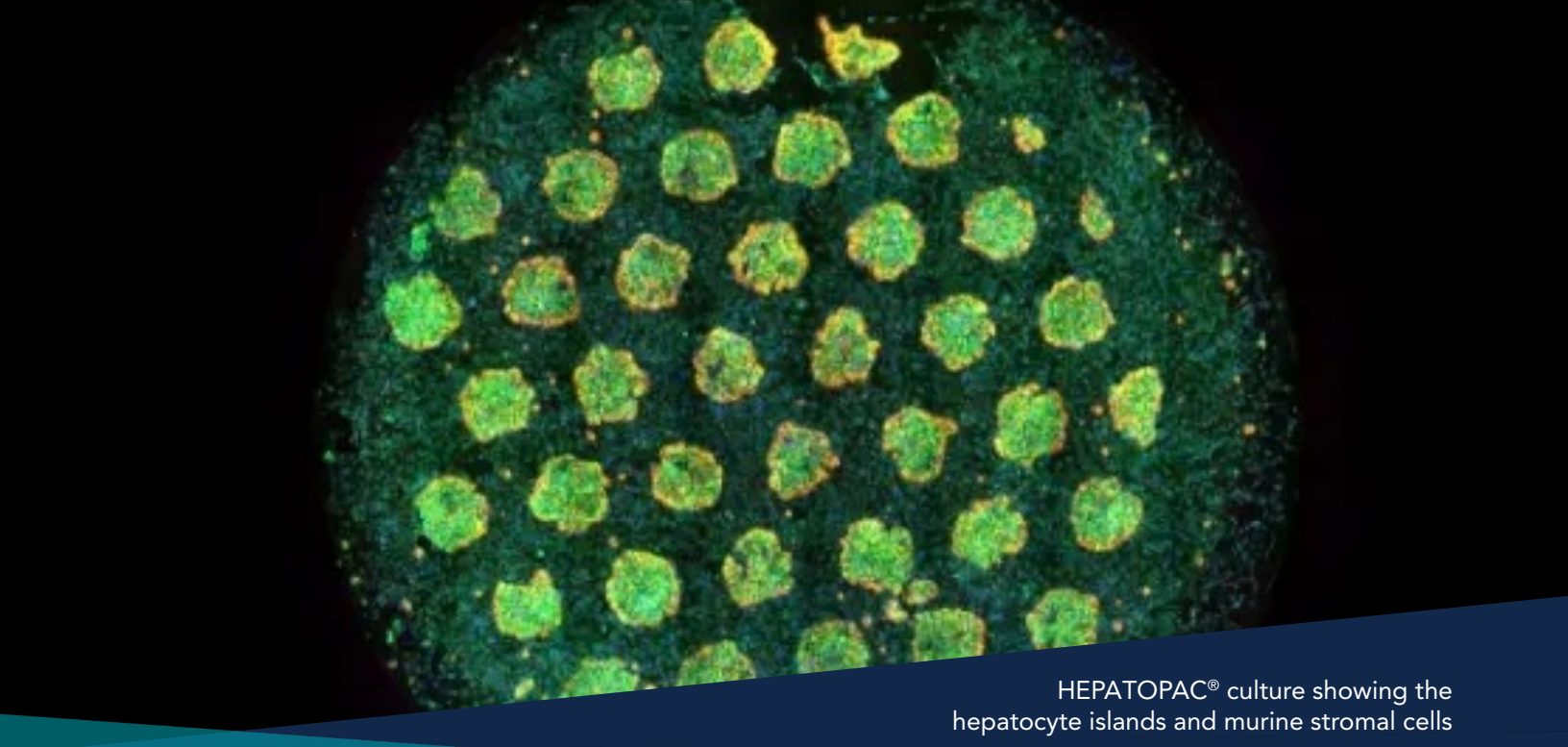
BioIVT offers Kupffer-enriched nonparenchymal liver cells and high-purity Kupffer cells. High-purity Kupffer cells are collected using an affinity purification method based on cell surface markers that preferentially attach to magnetic beads. They are cryopreserved immediately after isolation, ensuring the preservation of their phenotype. The purity of these cells is assessed by CD68 positivity using flow cytometry, and their function is evaluated based on interleukin 6 (IL-6) release in response to lipopolysaccharide (LPS) and phagocytosis of fluorescent latex beads.

BioIVT's high-purity Kupffer cells are prepared from whole livers on the same day as fresh human hepatocyte isolations. This process makes it possible to provide high-purity Kupffer cells and hepatocytes from the same donor in a custom product order.

Thawing and Plating

Human Kupffer cells should be thawed and plated using appropriate medium. For more information, read the Kupffer cells thawing and plating guide.

Learn more about high-purity Kupffer cells.



HEPATOPAC® culture showing the hepatocyte islands and murine stromal cells

Photo credit: BioIVT

4 Multicellular Systems

4.1. HEPATOPAC® Cultures

HEPATOPAC® technology creates stable *in vitro* liver models that enable long-term hepatic metabolism and toxicity studies. In the liver, hepatocytes are polarized and in direct contact with the blood supply and the bile canaliculi in configurations that are only one or two cells thick. HEPATOPAC cultures, formed using a proprietary patterning method, consist of hepatocyte "islands" surrounded with supportive stromal cells. This micropatterned design simulates the liver's *in vivo* architecture within the wells of industry-standard microtiter plates.

Hepatocytes can remain healthy and viable in the HEPATOPAC platform for over 4 weeks. They secrete albumin, synthesize urea, display functional bile canaliculi, and metabolize compounds using active phase I and II enzymes at higher levels than other *in vitro* liver models. These hepatocytes show better *in vitro–in vivo* correlations (IVIVC) than conventional hepatocyte models, especially for medium- and low-turnover compounds.

In addition to long-term metabolism and toxicity models, applications for HEPATAPAC cultures include reaction phenotyping, CYP inhibition studies, liver disease models (e.g., malaria, hepatitis, MASLD, and MASH) and gene therapy (e.g., siRNA transfection, GalNAc conjugated siRNA, and delivery via lipid nanoparticles).

HEPATOPAC plates are shipped as plated cells with the required media to culture the cells. Depending on the species, HEPATOPAC cultures can be configured in 24-, 96-, or 72-well plates. (A 72-well plate is a 96-well plate with the outer wells being acellular.) These cells can also be cultured in 96-well high-content imaging (HCI) plates.

Watch the HEPATOPAC instructional video

Read the HEPATOPAC instructions for use.

Learn more about human HEPATOPAC® cultures.

Species available in HEPATOPAC cultures include:

- Human: Single-donor or multidonor LIVERPOOL®
- Dog
- Monkey
- Mouse
- Rat
- Multispecies

For more information, visit HEPATOPAC FAQs.

4.2. HEPATOMUNE® Cultures

HEPATOMUNE® cultures are a triculture of human hepatocytes, stromal cells, and Kupffer cells in 24- and 96-well plate formats.

HEPATOMUNE cultures are created by adding Kupffer cells to HEPATOPAC cultures with a hepatocyte-Kupffer cell ratio of 1:0.4. HEPATOMUNE cultures model functional human liver tissue in inflammatory stress conditions and can be used to evaluate inflammation-mediated hepatotoxicity. This triculture also provides an optimal model for studying cytokines and cytokine modulators, which play an essential role in tissue repair, cancer development, cell replication, and other processes.

HEPATOMUNE cultures retain *in vivo*–like morphology, express liver-specific genes, metabolize compounds using active phase I/II drug metabolism enzymes, secrete liver-specific compounds, and exhibit transporter activity (phase III). The addition of primary Kupffer cells does not affect the functionality of the hepatocytes as determined by CYP3A4 activity and urea synthesis throughout a 10-day culture.

These cultures remain viable for at least 10 days (after receipt and recovery in researchers' labs) in industry-standard multiwell formats and are suitable for evaluating hepatotoxicity, monitoring protein therapeutic-drug interactions, and investigating reaction mechanisms.

Learn more about human HEPATOMUNE cultures.

Photo credit: BioIVT

5 Subcellular Fractions

5.1. Introduction

Subcellular fractions are widely used in drug discovery and preclinical drug development to evaluate species differences, metabolic activity, *in vitro* intrinsic clearance, reaction phenotyping, inhibition of CYPs and UGTs, and *ex vivo* enzyme induction.

BioIVT offers an extensive selection of tissue-derived subcellular fractions.

BioIVT's standard human subcellular products are outlined in **Table 6**.

Advantages of using BioIVT's subcellular fractions include:

- Large donor pools are used to minimize lot-to-lot variation and increase the long-term viability of each lot.
- BioIVT's experts follow standard operating procedures (SOPs) for the preparation and characterization of each lot to ensure consistency and quality.
- Processes used allow for the production of matching S9 and microsomal donor pools.
- V_{max} values are assayed for human pooled fractions.
- CYP and UGT activity characterization assays are performed.
- All lots must meet established acceptance criteria.

Table 6: Human Subcellular Products				
Tissue and subcellular product	Gender			Genotyped
	Female pooled	Male pooled	Gender pooled	
Liver microsomes	✓	✓	✓	✓
Liver S9	✓	✓	✓	
Liver cytosol	✓	✓	✓	
Intestinal microsomes			✓	
Intestinal S9			✓	
Intestinal cytosol	✓	✓	✓	
Kidney microsomes			✓	
Kidney S9			✓	
Kidney cytosol			✓	
Lung microsomes*			✓	
Lung S9*			✓	
Lung cytosol			✓	
Skin S9			✓	

*Smokers and nonsmokers

5.2. Human Microsomes

5.2.1 Liver Microsomes

BioIVT's human liver microsomes (HLM) are enriched for CYPs, flavin-containing monooxygenases, carboxylesterases, epoxide hydrolase, UGTs, and other membrane-bound, drug-metabolizing enzymes (DMEs). Microsomes are used to evaluate metabolic stability, metabolite formation, CYP inhibition, species comparisons, and intrinsic clearance and to identify enzymes contributing to a drug's metabolism. **Table 7** summarizes BioIVT's portfolio of HLM products.

Large donor pools (n=200) represent the average enzymatic activity present in the general North American population, while intermediate-sized donor pools (n=50) achieve specific activity parameters for common DMEs. Small pools, or individual donor HLMs, allow for greater individual donor variability to influence results.

Each donor is fully characterized, and pooled HLM lots include the following information:

- Cytochrome P450 content
- Cytochrome b5 content
- NADPH-cytochrome c reductase activity
- Common drug-metabolizing enzyme activity rates

Table 7: Human Liver Microsome Products

	XTreme™ 200-donor HLM	Gender pooled	Male pooled	Female pooled	Genotyped*
Donors	200	50	10	10	1
Gender	100 male 100 female	Male and female but pooled to achieve targeted enzymatic activity rather than equal numbers of male and female donors	Male	Female	Male and female lots
Donor representation	Each donor equally represented by tissue weight	Pooled to achieve targeted enzymatic activity rather than equal representation	Pooled to achieve targeted enzymatic activity rather than equal representation	Pooled to achieve targeted enzymatic activity rather than equal representation	NA
Intrinsic clearance data for major CYPs, UGTs, and FMOs	K_m V_{max}	V_{max}	V_{max}	V_{max}	V_{max}
CYP activity	Represents average American population	Slightly higher than general population	Donor-dependent	Donor-dependent	Donor-dependent
Standard volume available	10 mg 20 mg 100 mg 1000 mg	5 mg 20 mg 100 mg 1000 mg	10 mg	10 mg	10 mg

*Genotyping for CYP2C19, CYP2C9, CYP2D6, CYP3A5, UGT1A1, and UGT1A9 is available, with products rated as high, medium, or low expression.

5.2.2 Nonliver Human Microsomes

While the liver is the primary site of drug metabolism, other organs and tissues play an important role in drug ADME. Enzyme expression differences found in these organs can significantly affect the metabolism of specific drugs. Microsomes are used to assess these effects and the potential contribution to drug metabolism.

BioIVT offers pooled human microsomes from the following organs:

- Intestine
- Kidney
- Lung

Learn more and order human microsomes.

5.3. Liver S9

S9 fractions are a mixture of soluble cytosolic and microsomal enzymes and contain an array of phase I and II enzymes. Pooled S9 fractions are useful for assessing drug metabolism when the drug has the potential to be modified by both cytosolic and microsomal DMEs. Because S9 is a more comprehensive enzyme mixture, incubations supplemented with multiple enzymatic cofactors can be used to assess multiple DMEs in the same incubation. If multiple enzymes are predicted to metabolize the test compound, they may need to be tested separately for a more definitive understanding of each DME contribution.

Learn more about human S9.

5.4. Human Cytosol

Human cytosolic fractions contain a mixture of soluble DMEs (phase I and phase II) in which the membrane-bound microsomal enzymes have been depleted from the mixture by centrifugation. In the absence of the microsomal DME activities, the cytosol fraction makes it easier to understand the specific contribution of soluble DMEs because the microsomal enzymes are functionally depleted. Cytosol is made from the same starting material used for the production of S9 and microsomal products, ensuring consistency, reliability, and better correlation with patient profiles.

BioIVT offers standard cytosolic fractions isolated from the following tissues:

- Intestine
- Kidney
- Liver
- Lung

Learn more about human cytosol.

5.5 Reaction Phenotyping Kit

BioIVT's Reaction Phenotyping Kit is designed to assist in identifying human liver CYP and UGT enzymes responsible for metabolizing a drug or other xenobiotics. Human liver microsomes in the kit are carefully selected to minimize correlations or outliers that can interfere with reliable results by using donors with unique polymorphic enzyme activity profiles.

Learn more about reaction phenotyping kits.

Each kit contains:

- 16 individual samples of human liver microsomes
- Two vials of pooled human liver microsomes
- Comprehensive characterization data packet, including correlation tables for CYP, FMO, and UGT enzymes

5.6 Animal Subcellular Fractions: Microsomes, S9, and Cytosol

As summarized in Table 8, BioIVT offers animal subcellular liver microsomes, S9, and cytosol fractions for all major preclinical species. In addition, BioIVT can implement custom collections for nonstandard species.

Table 8: Animal Subcellular Fractions

Animal	Species	Gender			Tissue				
		Female pooled	Male pooled	Gender pooled	Liver	Lung	Kidney	Intestine	Skin
Mouse	CD-1 C57BL/6 BALB/C B6C3F1	✓	✓	✓	✓	✓	✓	✓	✓
Monkey, including CITES	Cynomolgus Rhesus	✓	✓	✓	✓	✓	✓	✓	
Rat	Sprague- Dawley Wistar Wistar Hannover	✓	✓	✓	✓	✓	✓	✓	✓
Dog	Beagle	✓	✓	✓	✓	✓	✓	✓	
Guinea Pig	Hartley	✓	✓	✓	✓	✓	✓	✓	
Hamster	Golden Syrian	✓	✓	✓	✓	✓	✓	✓	
Miniture pig	Göttigen Yucatan	✓	✓	✓	✓	✓	✓	✓	
Rabbit	New Zealand White	✓	✓	✓	✓	✓	✓	✓	

5.7 Cell Organelles

Human Liver Lysosomes

Human liver lysosomes are a site of degradation and catabolism within the cell. Lysosomes can be used to evaluate the stability and metabolism of biopharmaceuticals and macromolecules that are engineered to enter the target cell through the endosomal-lysosomal pathway.

Liver lysosomes can be useful in understanding and predicting off-target liver catabolism and potential hepatotoxicity, even if the hepatocytes are not the intended target cells. Purified lysosome fractions show superior lysosomal enzymatic activity compared to microsomes and S9 fractions with low contaminating activity from mitochondrial enzymes.

Learn more about human liver lysosomes.

Rat Liver Tritosomes

Rat liver tritosomes (lysosomes loaded with Tyloxapol) are also available. They exhibit decreased density and are more easily isolated from cellular organelles like mitochondria that have overlapping densities with native lysosomes. Rat liver tritosomes are a predictive *in vitro* test system with high specific activity used to investigate the catabolism of small- and large-molecule drugs fated to the endosome-lysosome pathway in humans or to study lysosomal composition, function, and disease. Mouse liver tritosomes can be prepared by the BioIVT custom products team.

Learn more about rat liver tritosomes.

Human Liver Mitochondria

Preparations of human liver mitochondria contain MAO-A and -B, aldehyde dehydrogenases, and other xenobiotic-metabolizing enzymes. Mitochondria are used to study compounds metabolized by mitochondrial enzymes or in studies of mitochondrial toxicity.

Learn more about human mitochondria.

6 BACTOSOMES® and Other Recombinant Enzymes

6. Introduction

BACTOSOMES® enzymes and other recombinant enzymes are helpful in test systems requiring high levels of individual enzymatic activities. Because these products are individual enzymes—rather than a mixture of enzymes, as exists in cell cultures or subcellular fractions—they can be used to determine the role of a specific enzyme in the metabolism of a xenobiotic.

The most common applications for recombinant enzymes are reaction phenotyping studies and enzyme inhibition studies. Additionally, recombinant enzymes are used in metabolite synthesis screening programs.

6.1 BACTOSOMES Enzymes

BACTOSOMES enzymes are bacterial membranes containing individual recombinant CYP enzymes coexpressed with NADPH P450 reductase. These enzymes are made by genetically modifying *E. coli* bacteria to express human or animal enzyme proteins. Bacteria are an ideal system because they do not natively express CYPs, so the chances of background activity are low. After the bacteria are incubated, the cells are lysed, and the enzyme-containing membranes are isolated.

BACTOSOMES enzymes are available for all the CYP isoforms used in preclinical drug development, including CYP1, CYP2, CYP3, CYP4, CYP17, CYP26, CYP46, and CYP51.

BACTOSOMES enzymes are available for all the species typically investigated in preclinical testing are available, including:

- Human
- Dog
- Monkey
- Mouse
- Pig
- Rat

BioIVT offers most BACTOSOME products in low-reductase and high-reductase formats, as well as with or without b5, which provides flexibility to fit into a wide range of assay formats. The system used to generate BACTOSOMES is effective for a broad range of enzymes, making it possible to implement custom syntheses of enzymes that are not included as standard products.

Most human CYPs are available in BioIVT's EasyCYP format. EasyCYPs have a standard CYP concentration, protein concentration, and vial size throughout the range, which makes EasyCYPs easy to use for most models.

The advantage of "regular" BACTOSOMES is that study designs can include more CYP per milligram of total protein, giving researchers more control. In a typical metabolism identification study requiring higher concentrations of CYPs, regular BACTOSOMES would likely be the preferred system.

Read the BACTOSOMES enzymes guide.

Learn more about BACTOSOMES enzymes.

Read the Human EasyCYP™ BACTOSOMES enzymes guide.

Learn more about EasyCYP™ BACTOSOMES.

6.2 Non-CYP Recombinant Enzymes

BioIVT also produces enzymes responsible for secondary metabolism using transfected *E. coli* cells or SF9 insect cells transfected with baculovirus. BioIVT's extensive catalog of non-CYP enzymes includes ALDH, AOX, CES, GST, MAO, SULT, and UGT.

Learn more about non-CYP recombinant enzymes.

Appendix I: Ordering Products

More information about BioIVT's extensive catalog of biospecimens can be found at Products | BioIVT. Hepatocytes, HEPATOPAC®, and HEPATOMUNE® products are listed on the website under Cell Products. Microsomes, S9, recombinant enzymes (BACTOSOMES®), and other products are listed under Subcellular Fractions & Recombinant Enzymes.

BioIVT's Business Development, Scientific Affairs, and Customer Experience teams provide expertise and advice on ADME products based on researchers' applications. For product assistance and placing orders, click BioIVT Contact Us (bioivt.com/about/contact-us).

Customers can set up a complimentary account on BioIVT's digital portal, where they can manage their entire order process, including:

- Search for products by category and SKU
- View product prices
- Access educational resources, reference documents, and product documentation
- View ready-to-ship inventory
- Generate a quote
- Order product
- View orders
- Duplicate a previous order
- View invoices

Learn more about how to set up an account on the BioIVT digital portal.

Contact information for BioIVT:

North America, Asia Pacific, Middle East, and Africa
T: 516-483-1196
E: customerservice@bioivt.com

Europe
T: + 44 (0) 1444 707333
E: cseurope@bioivt.com

Appendix II: Available Human Cryoplateable Hepatocytes

Product ID	Description	Gender	Donors	Cells/vial (millions)
F00995-P	Female human cryoplateable hepatocytes	Female	1	5
M00995-P	Male human cryoplateable hepatocytes	Male	1	5
X008001-P	LIVERPOOL® 10-donor human cryoplateable hepatocytes	Mixed	10	5
X008052-P	LIVERPOOL® 5-donor human cryoplateable hepatocytes	Mixed	5	5
F00995-CXP	Female human CryostaX® cryoplateable hepatocytes, individual	Female	1	5
M00995-CXP	Male human CryostaX® cryoplateable hepatocytes, individual	Male	1	5
X008001-CXP	Mixed-gender human CryostaX® 10-donor pooled cryoplateable hepatocytes	Mixed	10	5
X008052-CXP	Mixed-gender human CryostaX® 5-donor pooled cryoplateable hepatocytes	Mixed	5	5
F00995-TCERT	Female human cryoplateable hepatocytes, TRANSPORTER CERTIFIED®	Female	1	5
M00995-TCERT	Male human cryoplateable hepatocytes, TRANSPORTER CERTIFIED®	Male	1	5
F00995-SCERT	Female human cryoplateable hepatocytes, SPHEROID CERTIFIED™	Female	1	5
M00995-SCERT	Female human cryoplateable hepatocytes, SPHEROID CERTIFIED™	Male	1	5

Appendix III: Available Human Cryosuspension Hepatocytes

Product ID	Description	Gender	Donors	Cells/vial (millions)
F00995	Female human cryosuspension hepatocytes	Female	1	5
M00995	Male human cryosuspension hepatocytes	Male	1	5
X008052	LIVERPOOL® 5-donor human cryosuspension hepatocytes	Mixed	5	5
X008001	LIVERPOOL® 10-donor human cryosuspension hepatocytes	Mixed	10	5
FX008001	LIVERPOOL® 10-donor human cryosuspension hepatocytes	Female	10	5
MX008001	LIVERPOOL® 10-donor human cryosuspension hepatocytes	Male	10	5
X008000	LIVERPOOL® 20-donor human cryosuspension hepatocytes	Mixed	20	5
X008005	LIVERPOOL® 50-donor human cryosuspension hepatocytes	Mixed	50	5
X008200	LIVERPOOL® 200-donor human cryosuspension hepatocytes	Mixed	200	5
F00995-CX	Female human CryostaX® cryosuspension hepatocytes, individual	Female	1	5
M00995-CX	Male human CryostaX® cryosuspension hepatocytes, individual	Male	1	5
X008001-CX	Mixed-gender human CryostaX® 10-donor pooled cryosuspension hepatocytes	Mixed	10	5
X008000-CX	Mixed-gender human CryostaX® 5-donor pooled cryosuspension hepatocytes	Mixed	20	5

Appendix IV: Available Nonhuman Hepatocytes

Animal	Matrix	Species	Gender			Tissue				
			Female pooled	Male pooled	Gender pooled	Liver	Lung	Kidney	Intestine	Skin
Rat	Cryosuspension Cryoplateable	Sprague-Dawley Wistar Wistar Hannover Cryosuspension	✓	✓	✓	✓	✓	✓	✓	✓
Mouse	Cryosuspension Cryoplateable	CD-1 C57BL/6 BALB/C cryoplateable	✓	✓	✓	✓	✓	✓	✓	
Dog	Cryosuspension Cyoplateable	Beagle	✓	✓	✓	✓	✓	✓	✓	✓
Monkey	Cryosuspension Cyoplateable	Cynomolgus* Rhesus* Marmoset*	✓	✓	✓	✓	✓	✓	✓	
Guinea pig	Cryosuspension	Hartley	✓	✓	✓	✓	✓	✓	✓	
Minipig	Cryosuspension Cryoplateable	Göttigen	✓	✓	✓	✓	✓	✓	✓	
Rabbit	Cryosuspension	New Zealand Cryosuspension	✓	✓	✓	✓	✓	✓	✓	

*With or without cites permits