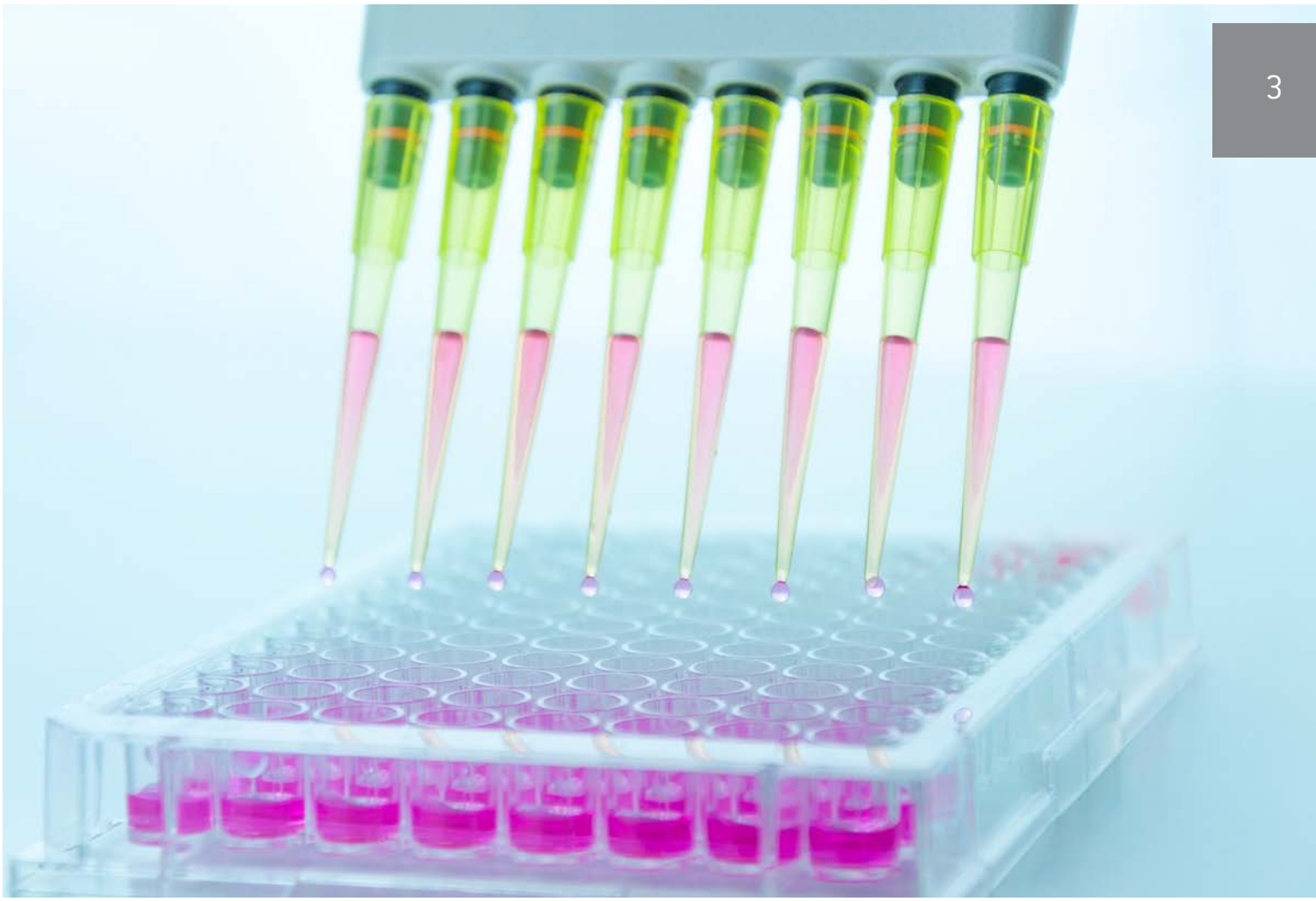
3 Hepatic Cells and Related Products



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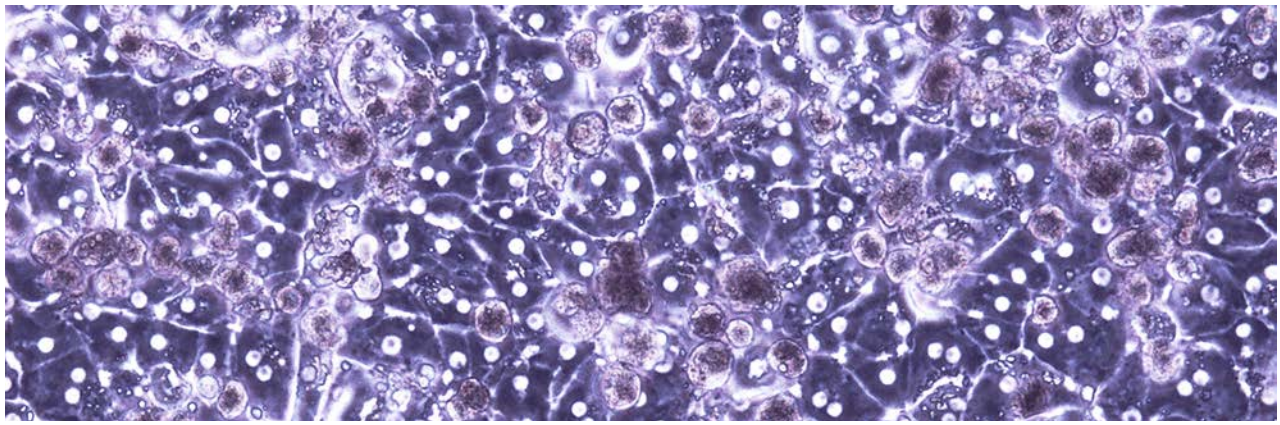


Human Hepatocytes

Introduction

Comprehensive ADMETox Portfolio

When conducting *in vitro* drug discovery and preclinical drug development research, the quality and the performance of the tools are critical to achieving physiologically relevant results. Triangle Research Labs (TRL), now part of Lonza, provides a toolbox of hepatic cell products supporting ADME, Toxicology, and discovery researchers globally. Our product portfolio includes primary hepatocytes, non-parenchymal hepatic cells, NoSpin HepaRG®, hepatic media, and Quasi Vivo® Cell culture systems.

The importance of the liver in physiology and disease.

The liver plays several critical roles in vertebrate biology. It performs numerous important metabolic functions, including regulation of glucose and cholesterol metabolism, the production of plasma proteins including clotting factors, and the detoxification of endogenous and exogenous compounds. The liver also produces various hormones involved in insulin regulation, blood pressure, and blood lipid levels. Because of the myriad of physiological processes that depend on the liver, having a fundamental understanding of liver biology and being able to address these at the benchtop is a key need for researchers involved in creating new life-saving medicines.

The liver is composed of five major cells types. The major cell type in the liver is the hepatocyte, which makes up about 70% of the liver cell population and is responsible for most of the metabolic and hormonal processes of the liver. The other four cell types collectively called hepatic non-parenchymal cells consist of resident macrophages known as Kupffers, stellates, liver sinusoidal endothelial cells, and cholangiocytes. These cells serve to support the structure of the liver, transport molecules in and out, and communicate with the immune system.

Isolated liver cells as an *in vitro* tool

When medicines are delivered orally, the liver extensively metabolizes them prior to systemic delivery. Because of this effect, the development process for most drugs must take into consideration the extent to which liver-specific metabolism will impact the short-term and long-term efficacy of the drug. Additionally, because the highest dose of any drug is present in the liver immediately after absorption, toxicity specific to the liver becomes an important factor when determining whether a drug can be advanced to market. In the last twenty years, isolated hepatocytes from human and animal livers have become a key tool for studying these effects during preclinical and non-clinical drug development programs. Both FDA and EMA Guidelines require certain metabolic tests to be performed using notably human primary hepatocytes for an *in vitro* evaluation of drug candidates.

When considering liver cells as disease models, there is increasing interest in understanding the role of both hepatocytes and the nonparenchymal cells as potential modulators of diabetes, fibrosis, liver injury, and infectious diseases.

TRL, now part of Lonza, has focused on developing products to support and facilitate more physiologically relevant *in vitro* research involving liver function and health.

Human Hepatocytes – Cryopreserved

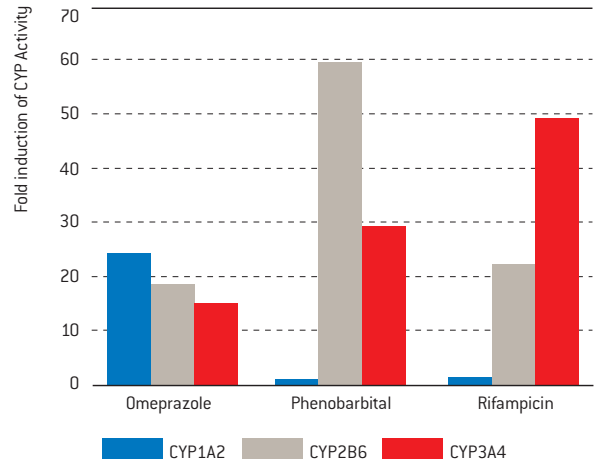
Our cryopreserved hepatocytes are isolated, cryopreserved, and characterized by our expert laboratory staff. The characterization is displayed on the certificate of analysis. Our cryopreserved hepatocytes must meet stringent standards to ensure optimal performance.

Source

- Lonza obtains tissues ethically through government regulated organ procurement organizations
- These tissues are non-transplantable whole livers or resected healthy liver tissues. Each tissue is procured in an ethical manner from consenting donors and is screened for infectious diseases including HIV, HBV, and HCV
- Basic donor demographics including age, gender, race, BMI, alcohol, tobacco, and drug use, relevant medical/ medication history are available for each donor, upon request

Applications

- Suspension and plated metabolism
- CYP450 induction
- Drug transporter assays
- Drug and chemical toxicity assays
- Xenotransplantation
- Cell therapy
- Disease modeling



Induction of major CYPs by prototypical inducers

Human Hepatocytes – Cryopreserved

Continued

Lot Qualification

Lonza charactrerizes cryopreserved material from each human donor to classify into one of 5 categories below. This pre-characterization provides researchers with important data endpoints to effectively select the right product for their needs.

- **Single Donor Cryopreserved Human Hepatocytes**
  - **Suspension Qualified** – Each lot specifically represents an individual and is qualified to be used only in short suspension assays (less than 4 hours). Specific CYP450 metabolism data and general Phase II metabolism data are available.

- **Pooled Cryopreserved Human Hepatocytes**
  - **Suspension Qualified** – These lots are produced by pooling cells together from multiple donors to represent an average population phenotype. Note that pooled human cryopreserved hepatocytes should be thawed with our pooled human cryopreserved hepatocyte medium (MCHT50P) for best results. Specific CYP450 metabolism data and general Phase II metabolism data are available.

- **Cryopreserved Human Hepatocytes,**
  - **Plateable, Metabolism Qualified** – These lots are derived from single donors and used for multiple applications, including the study of low-clearance compound metabolism. Each lot is tested for metabolic activity of known low-clearance substrates for CYP2C9, CYP2D6, and CYP3A4. These lots maintain confluence on a collagen-coated cell culture plate for a minimum of 3 days in culture.

  - **Plateable, Induction Qualified** – Used for a variety of applications, including to determine if compounds of interest are inducers of CYP1A2, CYP2B6, and/or CYP3A4. Qualification includes testing induction of each isoform with a known inducer and measuring the fold change in mRNA expression and CYP450 activity. These lots maintain confluence on a collagen-coated cell culture plate for a minimum of 5 days in culture.

  - **Plateable, Qualyst Transporter Certified**™ – Used for multiple applications including uptake and efflux transporter assays such as facilitated active uptake and B-Clear™. These lots are characterized for demonstration of transporter uptake and efflux activity similar to fresh human hepatocytes. Qualyst Transporter Solutions characterizes these cells for the expression and function of 9 different membrane specific transporters that play key roles in uptake and efflux of drugs. The cells are profiled following induction of CYP1A2, CYP3A4, and CYP2B6 enzymes. These lots are guaranteed to maintain confluence on a collagen-coated cell culture plate for a minimum of 7 days in culture.

Ordering Information – Cells

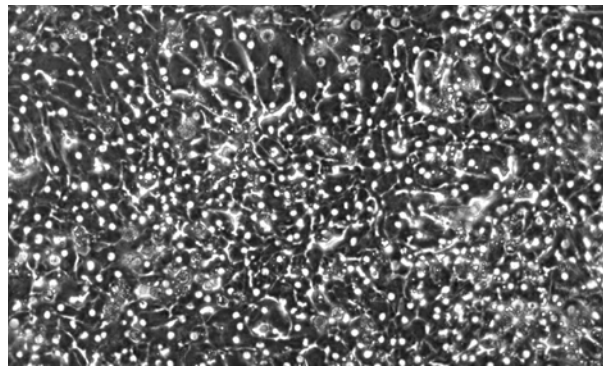
Cat. No. NA	Cat. No. EU	Product Name	Product Description	Size
HUCPI	HUCPI	Human Hepatocytes, Induction Qualified	Cryopreserved, plateable	≥5 million cells/vial
HUCPM	HUCPM	Human Hepatocytes, Metabolism Qualified	Cryopreserved, plateable	≥5 million cells/vial
HUCPQ	HUCPQ	Human Hepatocytes, Qualyst Transporter Certified™	Cryopreserved, plateable	≥5 million cells/vial
HUCSD	HUCSD	Human Hepatocytes	Cryopreserved, suspension	≥5 million cells/vial
HUCS10P	HUCS10P	Human Hepatocytes, 10 donor pool	Cryopreserved, suspension	≥5 million cells/vial
HUCS20P	HUCS20P	Human Hepatocytes, 20 donor pool	Cryopreserved, suspension	≥5 million cells/vial

Human Hepatocytes – Fresh

TRL offers freshly isolated human hepatocytes that are shipped to customers either in suspension or in monolayer culture on collagen-coated plates. Plate options include 6-, 12-, 24-, 48-, and 96-well plates, with or without extra-cellular matrix overlay.

Cell Characterization and Testing

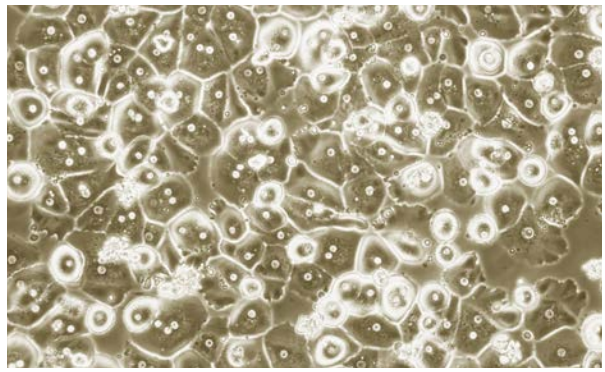
- Fresh human suspension hepatocytes are characterized for viability over 85%
- Fresh human plated hepatocytes are healthy and form confluent monolayers (over 85% confluence)
- To obtain freshly isolated human hepatocytes, sign up for our FreshAlert email, at www.lonza.com/products-services/bio-research/adme-tox/hepatocytes-and-media.aspx, to receive a notification for fresh human liver isolations. Each FreshAlert email includes a summary of the donor demographics, shipping schedule, and ordering instructions. The supply of fresh human hepatocytes is highly variable and dependent upon the availability of human liver tissue. Each donor of cells is tested and found non-reactive by an FDA-approved method for the presence of HIV-I, hepatitis B virus and hepatitis C virus. Where donor testing is not possible, cell products are tested for the presence of viral nucleic acid from HIV, hepatitis B virus, and hepatitis C virus.



Plated fresh human hepatocytes after 24 hours

Animal Hepatocytes – Cryopreserved

We aim to provide our customers with the highest quality hepatocytes on a schedule that can meet their demanding workload. Every week, we conduct multiple animal hepatocyte isolations. Sign up for our FreshAlert email, at www.lonza.com/products-services/bio-research/adme-tox/animal-hepatocytes.aspx, to receive a notification for fresh animal liver isolations. Our fresh animal suspension hepatocytes must be healthy with viability over 80%. Our fresh animal plated hepatocytes must be healthy and form confluent monolayers (over 80% confluence).



Plated cryopreserved mouse hepatocytes at 24 hours post-thaw

Standard Isolations Include the Following Species and Strains:

- Rat (Sprague-Dawley, Wistar, and Wistar Hannover)
- Mouse (CD-1 and C57Bl/6)
- Dog (Beagle)

Cryopreserved Animal Hepatocytes

TRL determines whether or not a lot is plateable (must exhibit 80% monolayer confluency for a minimum of 3 days in culture) and reports the metabolism data from the ethoxycoumarin O-deethylation assay. Phase I metabolism is represented by 7-ethoxycoumarin clearance, whereas Phase II metabolism is represented by the separate formation of 7-hydroxycoumarin glucuronide and 7-hydroxycoumarin sulfate from 7-hydroxycoumarin metabolism.

Lot Qualification

Suspension Applications

- All suspension qualified animal cryopreserved hepatocytes have post-thaw viabilities over 80%. These lots are also characterized for general Phase I and Phase II drug-metabolizing enzyme activity.

Plated Applications

- All plateable cryopreserved animal hepatocytes have post-thaw viabilities over 80%. Each lot can also be plated in a tissue culture collagen-coated plate or similar. These lots are guaranteed to maintain a healthy monolayer for a minimum of 3 days in culture. The monolayer confluency must be over 80% for the duration of the culture to be qualified as a plateable lot. These lots are also characterized for general Phase I and Phase II drug-metabolizing enzyme activity.

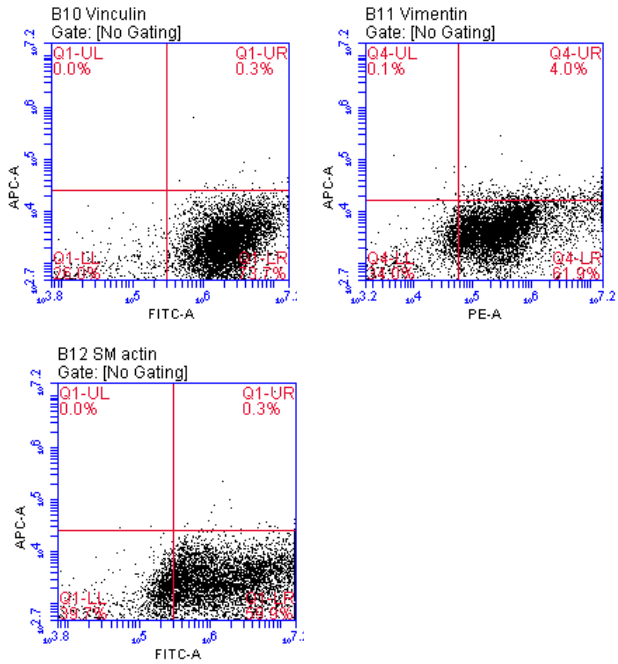
Ordering Information – Animal Cells

Cat. No. NA	Cat. No. EU	Product Name	Product Description	Size
RSCP01	RSCP01	Rat (Sprague Dawley) Hepatocytes	Cryopreserved, plateable	≥5 million cells/vial
RSCS01	RSCS01	Rat (Sprague Dawley) Hepatocytes	Cryopreserved, suspension	≥5 million cells/vial
RWCP01	RWCP01	Rat (Wistar Han) Hepatocytes	Cryopreserved, plateable	≥5 million cells/vial
RWCS01	RWCS01	Rat (Wistar Han) Hepatocytes	Cryopreserved, suspension	≥5 million cells/vial
RICP01	RICP01	Rat (Wistar) Hepatocytes	Cryopreserved, plateable	≥5 million cells/vial
RICS01	RICS01	Rat (Wistar) Hepatocytes	Cryopreserved, suspension	≥5 million cells/vial
MCCP01	MCCP01	Mouse (CD-1) Hepatocytes	Cryopreserved, plateable	≥5 million cells/vial
MCCS01	MCCS01	Mouse (CD-1) Hepatocytes	Cryopreserved, suspension	≥5 million cells/vial
MBCP01	MBCP01	Mouse (C57Bl/6) Hepatocytes	Cryopreserved, plateable	≥5 million cells/vial
MBCS01	MBCS01	Mouse (C57Bl/6) Hepatocytes	Cryopreserved, suspension	≥5 million cells/vial
DBCP01	DBCP01	Dog (Beagle) Hepatocytes	Cryopreserved, plateable	≥5 million cells/vial
DBCS01	DBCS01	Dog (Beagle) Hepatocytes	Cryopreserved, suspension	≥5 million cells/vial
CYCP01	CYCP01	Monkey (Cynomolgus) Hepatocytes	Cryopreserved, plateable	≥5 million cells/vial
CYCS01	CYCS01	Monkey (Cynomolgus) Hepatocytes	Cryopreserved, suspension	≥5 million cells/vial

Hepatic Non-Parenchymal Cells

Lonza provides human and animal, fresh hepatic non-parenchymal cells (NPCs) to support complex liver culture models and microtissues. Fresh Liver NPCs are typically available after each fresh hepatocyte (human and other animal) isolation. Sign up for our FreshAlert email, at www.lonza.com/products-services/bio-research/adme-tox/animal-hepatocytes.aspx, to receive a notification for fresh liver isolations.

Cryopreserved isolated liver Stellate cells and isolated liver Kupffer cells are also available. All cryopreserved lots are characterized by Flow Cytometry analysis utilizing specific cell markers and detailed in a cell data sheet.



Sample Flow cytometry data for Stellate cells. Vimentin, Vinculin, and SMA actin are markers used to characterize Stellate cells.

Ordering Information – Human Cells

Cat. No. NA	Cat. No. EU	Product Name	Product Description	Size
HUCLK	HUCLK	Human Kupffer Cells	Cryopreserved	≥1 million cells/vial
HUCLS	HUCLS	Human Stellate Cells	Cryopreserved	≥100,000 cells/vial

Silensomes™ HLM

Silensomes™ are human pooled-donor liver microsomes (HLM) irreversibly inactivated for one specific CYP using mechanism based inhibitors (MBI).

Each Silensomes™ HLM is available as cryopreserved, ready-to-use HLMs chemically knocked-out for one specific CYP activity (1A2, 2A6, 2B6, 2C8, 2C9, 2D6, , 3A4) with each showing high specificity and efficiency of their targeted CYP inhibition (>80%), and only minor impact (<20%) on other enzymes. The thaw and go format of Silensomes™ HLM enables researchers to focus more on results and less on validating the level of CYP inhibitions.

Ordering Information – Silensomes™ HLM

Cat. No. NA	Cat. No. EU	Product Name	Size
SIL000	SIL000	Master Control Silensomes™ HLM	1 vial
SIL200K	SIL200K	Silensomes™ CYP3A4 Kit with Control	2 vials
SIL-MCPNL	SIL-MCPNL	Silensomes™ CYP Phenotyping Kit	8 vials
SIL210K	SIL210K	Silensomes™ CYP1A2 Kit with Control	2 vials
SIL220K	SIL220K	Silensomes™ CYP2A6 Kit with Control	2 vials
SIL230K	SIL230K	Silensomes™ CYP2B6 Kit with Control	2 vials
SIL250K	SIL250K	Silensomes™ CYP2C8 Kit with Control	2 vials
SIL260K	SIL260K	Silensomes™ CYP2C9 Kit with Control	2 vials
SIL240K	SIL240K	Silensomes™ CYP2D6 Kit with Control	2 vials

Hepatic Media

TRL provides application specific hepatic media for the thawing, plating, and culture of fresh and cryopreserved, human and animal, primary hepatocytes. Each medium has been optimized and formulated for its function and species.

Ordering Information – Media

Cat. No. NA	Cat. No. EU	Product Name	Product Description	Size
MCAT50	MCAT50	Animal Hepatocyte Thawing Medium	For cells DBCP01, DBCS01	50 mL
MCHT50	MCHT50	Human Hepatocyte Thawing Medium	For cells HUCPI, HUCPM, HUCPQ, HUCSD, HUCPG	50 mL
MCHT50P	MCHT50P	Pooled Human Hepatocyte Thawing medium	For cells HUCS10P, HUCS20P	50 mL
MCRT50	MCRT50	Rodent and Monkey Hepatocyte Thawing Medium	For cells RSCP01, RSCS01, RWCP01, RWCS01, RICP01, RICS01, MCCP01, MCCS01, MBCP01, MBCS01, MXCP01, MXCS01, CYCP01, CYCS01	50 mL
MCST250	MCST250	Human Stellate Cell Growth Media	For cells HUCLS	250 mL
MM250	MM250	Hepatocyte Maintenance Media	For all hepatocytes	250 mL
CC-3198	CC-3198	HCM™ Hepatocyte Culture Medium BulletKit™	Includes basal medium and SingleQuots™ Kit	Kit
MP100	MP100	Hepatocyte Plating Medium	For all hepatocytes	100 mL
MP250	MP250	Hepatocyte Plating Medium	For all hepatocytes	250 mL
MCKM250	MCKM250	Human Kupffer Cell Maintenance Media		250 mL
MCKP250	MCKP250	Human Kupffer Cell Plating Media		250 mL

See pages 426–427.

NoSpin HepaRG™

NoSpin HepaRG™ cells are fully-functional, adult-phenotype, human hepatocyte like cells. The NoSpin HepaRG™ exhibits many characteristics similar to, and can be used for many of the same applications as, primary human hepatocytes. The NoSpin HepaRG™ is provided in a convenient, terminally differentiated, cryopreserved format that does not require the user to centrifuge, resuspend, or cell count after thawing. Just thaw and go! Each lot has consistent yield, viability, and functionality across multiple applications.

Applications:

- Short- and long-term hepatotoxicity
- Quantitative and qualitative metabolic analyses
- Drug-drug interaction potential
- Assessment of hepatic transporter interactions
- Disease modeling and antiviral research
- Alternative culture models

Features:

- High CYP450 activity for extended periods of time
- Complete expression of all nuclear receptors
- Similar to primary human hepatocytes in transporter and induction potential
- Fully characterized for post-thaw viability and yield, cell morphology, metabolic activity, induction potential, and transporter uptake activity

Ordering Information – Cells

Cat. No. NA	Cat. No. EU	Product Name	Product Description	Size
NSHPRG	NSHPRG	NoSpin HepaRG™ Cells	Cryopreserved	≥8 million cells/vial

Ordering Information – Media

Cat. No. NA	Cat. No. EU	Product Name	Product Description	Size
MH100	MH100	HepaRG™ Base Medium with Supplement	Serum-free base medium	100 mL
MHIND	MHIND	HepaRG™ Induction Medium Supplement	Base medium additive for induction assays	0.6 mL
MHMET	MHMET	HepaRG™ Maintenance/Metabolism Supplement	Base medium additive for metabolism assays	14 mL
MHPIT	MHPIT	HepaRG™ Pre-Induction and Tox Medium Supplement	Base medium additive for toxicity assays	12.5 mL
MHTAP	MHTAP	Thaw and Plating Medium Supplement	Base medium additive for thawing and plating	11.8 mL

See pages 426–427.

Quasi Vivo® Cell Culture Flow Systems

Quasi Vivo® technology provides an effective and simple-to-use method allowing for creation of more physiologically relevant models. Exposure to gentle medium flow stimulates gene expression and replenishes nutrients, maintaining peak cell viability and generating cell functionality closer to *in vivo*. The modular nature of the system allows multiple chambers to be connected in a variety of formats, facilitating more complex cellular and tissue interactions models.

QV900 with integrated Parker Polyflex peristaltic pump

Applications

Created by U.K. based Kirkstall, Ltd., Quasi Vivo® has been used to address a variety of research questions, including, but not limited to:

- Drug discovery and development
- Disease modeling
- Advanced organ models
- Cancer research
- Stem cell research

System Design

- Quasi Vivo® Systems are designed to be compatible with both 3D and 2D culture models with chamber diameters equivalent to a well of a standard 24-well plate. The systems can be readily set up in a standard biosafety cabinet and the entire pump and culture systems fit in standard humidified cell culture incubators.

QV500

- The QV500 is a flexible research tool optimized for cell and tissue culture use. Manufactured from medical grade silicone, the chamber forms a tight seal, allowing flow of media over cells without leakage or contamination issues. The modular design of the system allows the chambers to be set up in a wide array of configurations to best suit individual research needs and produce models with greater physiological relevancy than conventional *in vitro* techniques.

QV600

- The QV600 chamber provides an air/liquid interface for culture of cells that require contact with both air and medium to function properly, such as those in lung or skin tissues. The easy-to-use device is connected to a circulating medium supply through tubing and a peristaltic pump. The QV600 is compatible with cell culture inserts, scaffolds or tissue explants, and can be connected to other QV chambers for formation of systemic co-culture models with other cell types.

QV900

- The QV900 optical tray provides an ideal platform for higher throughput research. Produced from high-quality polypropylene, each tray holds six chambers, which can be connected together by media flow in any combination to suit your research needs. Flexible setup allows the QV900 to retain much of the modularity of the QV500 single chambers within an easy-to-handle format. Cells can be cultured directly onto the base of the chambers, allowing imaging of cells within the chamber, or on a 3D scaffold or cover slip, offering a range of options depending on your experimental needs.

Ordering Information – Cell Culture System

Cat. No. NA	Cat. No. EU	Product Name
QVCWSK	QVCWSK	QV500 Culture-well Starter Kit
QVTWSK	QVTWSK	QV600 Trans-well Starter Kit
QV6WSK	QV6WSK	QV900 6-well Starter Kit
QVPP2C	QVPP2C	Polyflex 2-channel peristaltic pump
QVPP6C	QVPP6C	Polyflex 6-channel peristaltic pump
QVRB08	QVRB08	Reservoir Bottle, 8 mL
QVRESB	QVRESB	Reservoir Bottle, 30 mL
QVRB125	QVRB125	Reservoir Bottle, 125 mL

North America – Customer Service: +1 800 638 8174 (toll free); order.us@lonza.com; Scientific Support: +1 800 521 0390 (toll free) scientific.support@lonza.com