Human Primary Hepatocytes
Product Overview

General Background
Hepatocytes represent nearly 80% of the total volume and 60% of the total number of cells in the average healthy human adult liver (Kmieć, 2001, Bioulac-Sage et al., 2007). They are specialized epithelial cells that exhibit a highly polarized architecture and organization of the cytoplasm and plasma membrane in order to fulfill the multitude of tasks that they are required to perform. They perform a majority of the biochemical and physiological functions associated with the liver, including the synthesis of key binding and carrier proteins in the blood (e.g., albumin, thyroxine-binding globulin [TBG], ceruloplasmin, transthyretin, insulin-like growth factor [IGF], retinol, and vitamin D binding proteins) and the major enzymes and transporter proteins involved in the uptake, metabolism, and efflux of xenobiotics and their metabolites (Rodés, 2007; Klaassen et al., 2013).

Inside the liver, there are unique microenvironments that are inherently created within the context of the liver’s native microanatomy within which hepatocytes reside in vivo. The liver acinus is demarcated into three zones: zone 1 is the perportal region; zone 2 is the midlobular region; and zone 3 is the pericentral region (Rappaport, 1977, Ito and McCuskey, 2007). Due to the particular location of hepatocytes along the microvasculature and the directionality of blood flow through the lobular units, they are exposed to natural gradients and microenvironments that control their gene expression profiles and phenotype (Smith and Wills, 1981, Ugele et al., 1991, Gebhardt, 1992). Matrix chemistry, solute concentrations, oxygen tension, and hepatocyte size and ploidy vary across the lobular microstructure, which results in distinct zonal differences in gene expression, cell phenotypes and functional capabilities (Probst and Jungermann, 1983, Wolfe and Jungermann, 1985, Wojcik et al., 1988, Reid et al., 1992, Lindros, 1997, Turner et al., 2011, Wang et al., 2011).

Examples of the differences in the zonal expression of genes in hepatocytes related to endo- and xenobiotic uptake and clearance have been highlighted in a number of review publications (LeCluyse et al., 2012; Godoy et al., 2013). Fatty acid β-oxidation, cholesterolic metabolic capacity and PPAR-regulated gene expression is greatest in zone 1 hepatocytes, whereas bile acid, lipogenic and glutamine synthase metabolic capacity is greatest in zone 3 hepatocytes. The highest levels of most cytochrome P450 (CYP) enzymes involved in the biotransformation of xenobiotics can be detected in hepatocytes located in zone 3, beginning with the cells surrounding the central veins and extending to approximately the midlobular region. The total number of hepatocytes expressing these genes is dependent, in part, on previous exposure to both endogenous and exogenous activators of nuclear receptors (e.g. enzyme inducers and metabolites of bile acids) and other regulators of uptake transporter and metabolizing enzyme gene expression (Wojcik et al., 1988, LeCluyse et al., 2012, Gebhardt and Matz-Soja, 2014). These regional differences in CYP expression are partially responsible for the zonal pattern of toxicity exhibited in vivo upon exposure to many bioactivated compounds, such as acetaminophen (APAP), carbon tetrachloride, bromobenzene, and chloroform (Black, 1984; Tomasi et al., 1985; Anundi et al., 1993; Moon et al., 2010).

Many important uptake transporter proteins are also expressed in a zone-specific fashion. For example, human liver sections stained with antibodies against human OATP1B1 and OATP1B3 exhibit a similar pattern as that for CYP3A4 in zone 3 hepatocytes. The overlapping expression of both transporter and...
CYP proteins in zone 3 cells suggests that hepatocytes acquire the ‘mature’ phenotype with the full capacity to accumulate and metabolize xenobiotics somewhere during the zone 2 transition, which also makes them more susceptible to toxicities associated with CYP-mediated reactive metabolites. By contrast, zone 1 hepatocytes do not appear to be impacted by these types of events. However, in the case of other liver toxicants (e.g., allyl alcohol, phosphorus), zone 1 specific toxicity may be observed as a result of the unique oxygen, biotransformation, and cytokine-mediated effects located near the portal triad (Badr et al., 1986; Przybokci et al., 1992).

Utility of primary human hepatocytes for ADMET applications

In the pharmaceutical industry in vitro hepatocyte culture strategies have proven useful during drug development for studying drug metabolism and drug-drug interactions before in vivo proof-of-concept studies are initiated (Hughes et al., 2011; Astashkina et al., 2012). As generally accepted, primary hepatocyte-based in vitro models are considered the gold standard for assessing the metabolism and enzyme induction potential of a new chemical entity. For example, in vitro testing strategies using primary human hepatocytes in suspension culture have been applied successfully to predict the in vivo pharmacokinetics and clearance of compounds for years, or using them in a sandwich culture (SC) model system for assessing the potential of compounds to be involved in significant adverse interactions through the induction or inhibition of liver enzymes (Lin, 2006; Hewitt et al., 2007; Obach, 2009; Obach et al., 2008). These sandwich cultures also have proven useful for the study of drug uptake and efflux transporters, and predicting the biliary secretion of compounds and their metabolites (Swift et al., 2010).

In the event that mechanisms of hepatotoxicity are being investigated, in vitro model systems using primary human hepatocytes allows for the rapid testing of larger sets of drug candidates — such as assessing the formation of reactive metabolites which can complex with cellular proteins (protein adducts), perturbation of mitochondrial function, or inhibition of key transport efflux proteins — among the many factors that have been shown to be important contributing factors to Drug Induced Liver Injury (DILI) (Evans et al., 2004, Pedersen et al., 2013). Other studies have confirmed that much greater fidelity with clinical outcomes is achieved using hepatocyte-based assays, especially where species differences, metabolites or other major pathways of uptake or efflux are involved in the disposition of drugs and bile acids (Oorts et al., 2015; Yang et al., 2015; Yang et al., 2016).

More recently, new hepatocyte culture systems which are longer lasting and more metabolically stable have proven valuable to study both drug metabolism and transport as well as DILI in vitro (Ramsden et al., 2014; Ballard et al., 2016; Khetani et al., 2013). In addition, these more stable, long-term primary hepatocyte culture models are particularly useful to addressing long term clearance of drugs with low turnover rates (Lin et al., 2016). Improvements towards a more physiologically-relevant culture system has been accomplished by culturing hepatocytes in three-dimensional (3D) spheroid structures, which also allow long-term culture with greater metabolic stability. Recent publications have shown that such in vitro models have the potential to detect in vivo liver toxicities not observed with 2D monolayers (Messner et al., 2013). Both the SC and spheroid 3D culture approaches have significantly improved stability and longevity of liver-like in vitro properties as well as the predictivity when used as a model for DILI (Sistare et al., 2016).

Current isolation practices and the impact on hepatocyte profiles

Most protocols utilized today for the isolation of primary human hepatocytes are based on the original 2-step methods developed originally by Berry and Friend (1969) and Seglen et al. (1976) and rely on the use of crude, ill-defined preparations of collagenase and non-specific proteases. In addition, further enrichment using Percoll density gradient centrifugation is universally used almost without exception (Kreamer et al., 1986; LeCluyse et al., 1996). These methods when adapted for the isolation of primary human hepatocytes (LeCluyse et al., 2005; LeCluyse and Alexandre, 2010) produce batches of cells that vary in their composition and phenotype as they represent a mixture of zonal hepatocyte subpopulations with varying amounts of ‘contaminating’ non-parenchymal cells (NPCs).

LifeNet Health® (LNH) hepatocytes are procured under the most state-of-the-art conditions which utilize enhanced tissue flushing and preservation methods, which minimize warm and cold ischemia times, including the time from recovery to laboratory. The overall impact of these additional measures, which are unique to this industry, on the overall success rates of tissue processing and the quality of the hepatocyte preparations is immense, and represents a new standard for quality and performance. Moreover, the care and quality control measures that have been established for the production and characterization of LifeNet Health’s clinical products, which have been developed over the 35+ year history of LNH, are also brought to bear in its preclinical tissue and cell products. This new level of recovery and testing procedures under higher QC standards represents a new era for hepatocyte quality and performance that has been missing from this industry for the past decade.

Each batch of cryopreserved hepatocytes is carefully characterized to determine the post-thaw viability and yields per vial, as well as the morphological integrity and suitability for cell culture applications. In addition,
LifeNet Health's certificate of analysis (CoAs) for each batch of cryopreserved human hepatocytes contains all the relevant donor demographics, blood chemistries, BMI, pertinent drug history and serological test results. If needed, LifeNet Health can provide histological data (H&E, trichrome staining) and pathological reports for each donor tissue upon request. All CoAs also contain complete post-thaw reports that include the average yield and viability per vial, plus the recommended seeding density for obtaining optimal confluency for plateable lots (based on a 24-well plate format). The CoAs also contain specific activity data for the major CYP enzymes (reported as pmol/min/M viable cells) and phase II enzyme activity using 7-EC and 7-HC as probe substrates. In addition, LifeNet Health’s CoA’s contain the corresponding induction data (i.e. fold change for CYP1A2, CYP2B6 and CYP3A4 mRNA and specific activities) for all induction-qualified plateable batches of cryopreserved hepatocytes after treatment with omeprazole, phenobarbital and rifampin, respectively.

Subcategories of cryopreserved hepatocyte lots

After completing the post-thaw assessment, each batch of cryopreserved hepatocytes is categorized according to several criteria for general or specific applications. The following are the major classes of cryopreserved hepatocytes that currently are provided for your convenience. Please inquire for additional options or categories that are not listed below:

a) Adult Suspension/Metabolism Qualified:

Primary adult human hepatocytes are considered the ‘gold standard’ for determining the metabolic stability and metabolism of new compounds in development. Metabolic stability refers to the susceptibility of compounds to biotransformation and provides measures of intrinsic clearance from which secondary pharmacokinetic parameters, such as bioavailability and half-life, can be calculated when other data on volume of distribution and fraction absorbed are available (Obach et al., 2008; Obach, 2009). In the early phases of drug discovery, new chemical entities cannot be administered to humans; hence, predictions of these properties using in vitro cellular/subcellular fractions are the method of choice. LifeNet Health’s suspension batches of cryopreserved hepatocytes have been characterized for CYP enzyme activity using prototype selective substrates for CYP1A2, CYP2B6, CYP2C9, CYP2C8, CYP2C19, and CYP3A4 (testosterone/midazolam), as well as for combined phase I and II metabolism of 7-ethoxycoumarin (7-EC) to 7-hydroxycoumarin (7-HC) and the corresponding sulfated and glucuronidated conjugates. LifeNet Health’s CoAs contain all post-thaw results including average viability and yield per vial and representative images of the cells in suspension culture.

b) Adult Plateable/Induction Qualified:

In vitro induction studies are conducted to assess the potential for drug interactions in vivo. These in vitro studies attempt to evaluate the potential for a new chemical entity (NCE) to alter the metabolism of other drugs by increasing their clearance through upregulating phase I and II enzymatic reactions as well as uptake and efflux clearance mechanisms. Most drug-drug interactions that occur due to increased clearance are mediated through the activation of one or more nuclear receptors (NR) that upregulate the expression of enzymes and transporters involved in the clearance of drugs and other xenobiotics, such as aryl hydrocarbon receptor (AhR), pregnane-X-receptor (PXR), and constitutive androstane receptor (CAR). Use of cultured primary human hepatocytes has become the accepted gold standard for conducting in vitro testing of new drugs for their potential to reduce the efficacy of co-administered compounds or be involved in potentially harmful drug-drug interactions due to induction of hepatic clearance pathways. Moreover, data from in vitro hepatocyte studies that demonstrate there is unlikely to be an interaction can be used to rationalize and streamline the clinical drug-drug interaction program.

LifeNet Health®’s induction-qualified batches of cryopreserved hepatocytes have been tested for response to prototype inducers of select target genes of each NR pathway, namely omeprazole (CYP1A2), phenobarbital (CYP2B6), and rifampicin (CYP3A4), after 48 and 72 hour treatment and measurement of the fold change in both mRNA and enzymatic activity, respectively. LifeNet Health’s cells also have been characterized for CYP enzyme activity using prototype selective substrates for CYP1A2, CYP2B6, CYP2C9, CYP2C8, CYP2C19, and CYP3A4 (testosterone/midazolam), as well as for combined phase I and II metabolism of 7-ethoxycoumarin (7-EC) to 7-hydroxycoumarin (7-HC) and the corresponding sulfated and glucuronidated conjugates. LifeNet Health’s CoAs contain all donor information, post-thaw results including average viability and yield.
per vial, recommended seeding density for optimal monolayer formation, and representative images of the cell monolayers after 5 days in culture. All of LifeNet Health's prequalified lots are guaranteed to produce stable confluent monolayers for a minimum of 1 week and meet or surpass its induction specifications when used in conjunction with LifeNet Health's recommended culture conditions.

c) Adult High BMI/NAFLD/NASH:

Non-alcoholic fatty liver disease (NAFLD) is the most common chronic liver disease in Western countries with a wide disease spectrum. It ranges from the hepatic accumulation of lipids known as steatosis, to non-alcoholic steatohepatitis (NASH) wherein steatosis is accompanied by inflammation and fibrosis, or it can further progress to cirrhosis and hepatocellular carcinoma. NAFLD is defined by the presence of steatosis in more than 5% of hepatocytes in livers from patients with little or no alcohol consumption and it is frequently associated with obesity, lipotoxicity, insulin resistance, hyperglycemia, hypertension, and dyslipidemia (Anderson and Borlak, 2008; Lewis and Mohanty, 2010; Cohen et al., 2011). The progression of NAFLD to non-alcoholic steatohepatitis and cirrhosis, and ultimately to carcinomas, is governed by interplay of pro-inflammatory pathways, oxidative stress, as well as fibrogenic and apoptotic cues (Lickteig et al., 2007).

As the liver is the major organ of biotransformation, perturbations in hepatic signaling pathways due to NAFLD and other related diseases have effects on both xenobiotic and endobiotic metabolism (Fisher et al., 2009; Wree et al., 2011; Naik et al., 2013). Several major nuclear receptors involved in the transcription and regulation of phase I and II drug metabolizing enzymes and transporters also have endobiotic ligands including several lipids (Lee et al., 2008; Masson et al., 2008). Studies have shown that the presence of steatosis, oxidative stress and inflammatory mediators like TNF-α and IL-6 lead to alterations in nuclear receptor signaling, such as CAR, PXR, PPARα (Bhalla et al., 2004; Zhou et al., 2006; di Masi et al., 2009; Dong et al., 2009; Gao et al., 2009). These factors result in altered expression and activity of drug metabolizing enzymes (DMEs) or transporters (Naik et al., 2013; Cobbina and Akhlaghi, 2017). Existing evidence suggests that NAFLD has effects on CYP3A4, CYP2E1 and MRP3, whereby CYP3A4 activity is down-regulated in NASH and the activity of CYP2E1 and the efflux transporter MRP3 is up-regulated (Weltman et al., 1998; Donato et al., 2006, 2007; Varela et al., 2008; Aubert et al., 2011). As such, this growing body of evidence has made it clear that the alterations associated with NAFLD could be a potential source of drug variability in patients and could have serious implications for the safety and efficacy of xenobiotics (Barshop et al., 2011). As such, primary hepatocytes from patients with clinical manifestations of varying stages of NAFLD and NASH serve as an important research tool for the discovery of new drug targets and a better understanding of the clearance and toxicity of drugs in this growing patient population (Browning and Horton, 2004).

Because of LifeNet Health’s unique network of partner institutions, LNH has access to a broader range of tissues representing both healthy and diseased individuals. Accordingly, LNH is able to provide primary hepatocyte lots from donors with various stages of NAFLD, including steatosis, NASH and hepatocellular carcinoma (HCC). A histopathological evaluation of formalin-fixed, paraffin-embedded tissue sections after H&E and trichrome staining is performed by a certified liver pathologist. Primary hepatocytes that have been isolated from NAFLD livers are produced under LNH's proprietary procurement and isolation conditions using best practices and leading-edge methods for recovery, isolation and preservation. All cryopreserved batches of cells are carefully characterized for quality, plateability and functionality. LNH supplies both suspension and plateable batches of hepatocytes from both healthy and diseased organs, and provides CYP activity data for CYP1A2, CYP2B6, CYP2C9, CYP2C8, CYP2C19, and CYP3A4 (testosterone/midazolam), as well as data for the combined phase I and II metabolism of 7-ethoxycoumarin (7-EC) to 7-hydroxycoumarin (7-HC) and the corresponding sulfated and glucuronidated conjugates. LNH's CoAs contain all donor information, post-thaw results including average viability and yield per vial, recommended seeding density for optimal monolayer formation where applicable, and representative images of the cell suspensions or monolayers after 5 days in culture. For diseased tissues, representative histological sections after H&E and trichrome staining and a pathology report can be provided with each CoA upon request.
d) Neonatal/Pediatric/Juvenile:

Developmental changes in the expression of drug metabolism and other clearance mechanisms determine the pharmaco- and toxicokinetics of chemicals at different life stages. These differences are critical in regulating the clearance and accumulation of drugs, and thus influence the pharmaco- and toxicodynamic responses in newborns and children. Early research studies have shown that the ontogeny of drug metabolizing enzymes and uptake/efflux transport proteins as the underlying mechanisms for altered drug disposition during liver development (de Wildt et al., 1999; Hines, 2006, 2007; Richard et al., 2001; Strassburg et al., 2002; Peng et al., 2012; Cui et al., 2012). With the evolving knowledge on drug transporters in liver and other essential drug-processing organs, it is important to have access to human hepatocytes to study the age-related differences in metabolism and transport function, as well as to investigate the impact these may have on the potential of hepatotoxic events (Hines, 2006; Klaassen and Aleksunes, 2010).
LifeNet Health® Primary Human Hepatocyte Part Numbers

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<td>MTOXH1000</td>
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<td>Cryopreserved Primary Human Hepatocytes Juvenile</td>
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LifeNet Health® Media and Supplements

Media and supplements are available for culturing primary human hepatocytes.

Advantages and Benefits:

- Optimized for plating and culturing primary human hepatocytes
- Reliable and consistent performance across cell lots
- Easy media preparation – just thaw, mix and use
- Superior performance and consistent results in assays for drug metabolism, transporter activity, hepatotoxicity and CYP enzyme induction

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References


65. Smith, M. T. and Wills, E. D. Effects of dietary lipid and phenobarbital on the distribution and concentration of cytochrome-


