GIBCO™
Invitrogen Corporation

# THE HEPATOCYTE PRODUCT LINE

***Media for the Isolation and Culture of Adult Hepatocytes and Expression of Cytochrome P450***

**Liver Perfusion Medium**
**Cat. No.: 17701 500 mL**
**Storage Conditions: 2 to 8°C, in the dark.**

A buffered balanced salt solution formulated for cleansing the liver of blood, prevent clotting and to initiate the loosening of cell to cell contact. This medium is supplied antibiotic-free and should be supplemented with 10 mL (2X) of penicillin-streptomycin (Cat. No.15140) prior to use.

**Liver Digest Medium**
**Cat. No.: 17703 500 mL**
**Storage Conditions: -5 to -20°C, in the dark.**

A qualified Collagenase-Dispase medium for the dissociation of viable liver cells. This medium is supplied antibiotic-free and should be used within 24 hours of thawing overnight in a refrigerator (2-8°C).

**Hepatocyte Wash Medium**
**Cat. No.: 17704 500 mL**
**Storage Conditions: 2 to 8°C, in the dark.**

An enriched Williams' Medium E for the reduction of cell membrane leakage, ease of centrifugation and maintenance of viability. We recommend supplementing this medium with 5 mL of penicillin-streptomycin prior to use.

**HepatoZYME-SFM**
**Cat. No.: 17705 500 mL**
**Storage Conditions: 2 to 8°C, in the dark.**

**CAUTION:** **Human origin materials are non-reactive (donor level) for anti-HIV 1 & 2, anti-HCV, and $HB_sAg$. Handle in accordance with established bio-safety practices.**

This is a serum-free medium for the long-term maintenance of hepatocyte phenotypic expression including the active and inducible forms of Cytochrome P450 and active phase II enzymes. This medium should be supplemented with 2 mM L-glutamine and 5 mL (1X) penicillin-streptomycin prior to use.

## FEATURES

The Hepatocyte Product Line
- Transports the liver without ischemic damage
- Media that maximizes viable cell yields
- Maintains the xenobiotic metabolic activity of primary hepatocytes in culture

## Introduction

Hepatocytes play a major role in a variety of metabolic processes. After absorption from the gut, chemicals are transported to the liver where they may be converted to pharmacologically active or toxic metabolites or to hydrophilic excretable conjugates. Important components of the enzyme system needed for the metabolism of xenobiotics are a group of hemoproteins collectively known as the cytochrome P450 (CP450) system which function by oxidative metabolism. A characteristic property of this enzyme system is its inducibility by many chemicals or lipophilic substances associated with enhanced microsomal oxidation[1,2]. These inducers can be divided into three broad classes typified by drugs like phenobarbital, polycyclic hydrocarbons like benzo(a)pyrene and steroids such as testosterone[3,4].

Other than the problems normally associated with mammalian cell culture, a major limitation of rodent hepatocytes in traditional serum-supplemented culture has been the rapid loss (90% by day 2) of CP450 catalyzed Phase I reactions (oxidation, hydroxylation, reduction)[5]. Attempts to overcome this problem have focused on the culture of hepatocytes on specialized matrices, media formulations and media supplementation[6]. Invitrogen scientists have focused on the optimization of a media family for the isolation, culture and phenotypic expression of hepatocytes. The purification of viable hepatocytes is achieved using either Nycodenz or Percoll density gradients.
Hepatocyte serum-free medium has been compared to both serum-supplemented and serum-free controls using either EHS or Collagen I as a matrix. Results have repeatedly shown a significant improvement in the maintenance and inducibility of the two measured enzymes, ethoxycoumarin de-ethylase and benzo-a-pyrene hydroxylase.

## Quality Control Testing

All media have been demonstrated to support hepatocyte applications by performance testing using rat liver perfusion, digestion, culture and metabolic activity measurement. Standard testing for the product range includes pH, osmolality and tests for the absence of bacterial and fungal contaminants.

**Instructions for using the Hepatocyte Products**

**NOTE: Please refer to description of products for respective media supplementation.**

1. Adult Sprague-Dawley male rats (300 to 350 gms) are anesthetized with Ketamine and xylazine (60:5 mg/kg) given intramuscularly or with chlorohydrate (345 mg/kg).
2. The liver is isolated *in situ* and perfused with 350 mL of warm (37°C) **Liver Perfusion Medium** through the abdominal aorta (ligated below the kidneys and above the diaphragm) at a rate of 35 mL/minute with the perfusate exiting through the severed vena cava.
3. This is followed by a Collagenase-Dispase digestion with **Liver Digest Medium** at a rate of 35 mL/minute. This results in blanching, softening and dissociation of liver tissue and provides complete digestion of the liver in 10-12 minutes.
4. The liver is aseptically removed to a sterile 50 mL conical tube containing 15mL cold **L-15 Medium** and transferred to the cell culture laboratory on ice.
5. The hepatocytes are released by gently using a cell scrapper and pipetting with a large bore pipette. The cell suspension is spun down at 50 g for 5 minutes, resuspended in wash media and filtered through a sterile 100 µm nylon meshinto a 50 mL conical tube placed on ice, sedimented by centrifugation at 50 g for 5 minutes, resuspended and washed 2-3 times in 50 mL cold **Hepatocyte Wash Medium**.
6. Hepatocytes are purified by centrifugation on either 20% NYCODENZ® or alternatively by Percoll density gradient separation and washed twice more before being resuspended in the attachment medium. Either of these separation procedures give a cell yield of about 2.5 x $10^8$ cells with 90-95% viability, as determined by trypan blue exclusion.
7. Approximately 10 x $10^6$ cells in 25 mL of **Williams' Medium E,** supplemented with 5mL penicillin-streptomycin and either 5µg/$cm^2$ bovine fibronectin or 5% FBS are plated in 150-$cm^2$ tissue culture flasks precoated with a Collagen 1[7]matrix (12.5 µg/$cm^2$) or with the EHS matrix[8] (100 µg/$cm^2$ Matrigel, Collaborative Research Inc., Bedford, MA) and incubated in a humidified atmosphere of 5% $CO_2$ in air at 37°C. Collagen or EHS is diluted in ice cold 1X HBSS (Cat. No. 14170) and 20 mL/T160 $cm^2$ flask added, spread over the surface and incubated overnight at 37°C. The flasks should be washed with the Wash Medium prior to adding the cell suspension.
8. Unattached cells are poured off 2-3 hours after plating and replaced with 25 mL **HepatoZYME-SFM** supplemented with 1.25 µg/$cm^2$ rat tail Collagen to provide a sandwich matrix[9]. Cultures are refed with Hepatocyte SFM (without collagen) at 24 hours and every 48 hours thereafter. Hexobarbital (0.5-2.0mM) or other inducing agents may be added to cultures 48 hours before harvesting the cells.

***Analytical Procedures*:** The cells are harvested by scraping into ice-cold 100 mM $KPO_4$ buffer (pH 7.4) sonicated and fractionated to yield a microsomal pellet that may be frozen at -70°C prior to use. The pellet is homogenized in a buffer containing protease inhibitors and used for protein, Western blot and enzyme analysis.



**References:**


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