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life technologies

Hepatocyte Media

Media for the isolation and culture of adult hepatocytes and expression of Cytochrome P450

Description

Liver Perfusion Medium is a buffered, balanced salt solution formulated for cleansing the liver of blood, to prevent clotting, and to initiate the loosening of cell–to–cell contact.

Liver Digest Medium is a qualified Collagenase-Dispase medium for the dissociation of viable liver cells.

Hepatocyte Wash Medium is an enriched Williams' Medium E for the reduction of cell membrane leakage, ease of centrifugation, and maintenance of viability.

HepatoZYME-SFM 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.

Product	Catalog no.	Amount	Storage	Shelf life*
Liver Perfusion Medium (1X), liquid	17701-038	500 mL	2°C to 8°C; Protect from light	12 months
Liver Digest Medium	17703-034	500 mL	–20°C to –5°C; Protect from light	12 months
Hepatocyte Wash Medium	17704-024	500 mL	2°C to 8°C; Protect from light	12 months
HepatoZYME-SFM	17705-021	500 mL	2°C to 8°C; Protect from light	12 months

* Shelf Life duration is determined from Date of Manufacture.

Product use

For Research Use Only. Not for use in diagnostic procedures.

Important information

Hepatocyte media are supplied antibiotic free and should be supplemented with penicillin-streptomycin before use.

Safety information

Read the Safety Data Sheets (SDSs) and follow the handling instructions. Wear appropriate protective eyewear, clothing, and gloves.

Prepare media

Liver Perfusion Medium,

Aseptically add 10 mL of penicillin-streptomycin (100X) solution to 500 mL Liver Perfusion Medium, 2X final concentration before use.

Hepatocyte Wash Medium

Aseptically add 5 mL of penicillin-streptomycin (100X) solution to each 500 mL of Hepatocyte Wash Medium, 1X final concentration before use.

HepatoZYME-SFM

Aseptically add 5 mL of penicillin-streptomycin (100X) solution, and 5 mL of L-glutamine (200 mM) to each 500 mL of HepatoZYME-SFM, before use.

Liver Digest Medium

Thaw overnight in a refrigerator (2°C to 8°C). Liver Digest Medium is supplied antibiotic-free and should be used within 24 hours of thawing. **Do not** subject to additional cycles of freeze-thawing.

Use

Isolate liver

1. Anesthetize adult male rats (300–350 g) with Ketamine and xylazine (60:5 mg/kg) given intramuscularly or with chlorohydrate (345 mg/kg).
2. Isolate the liver *in situ* and perfuse (35 mL/minute) with 350 mL prewarmed (37°C) Liver Perfusion Medium through the abdominal aorta. Ligate below the kidneys and above the diaphragm with the perfusate exiting through the severed vena cava.
3. Perfuse (35 mL/minute) liver as in step 2 of this procedure with Liver Digest Medium. Collagenase-Dispase digestion results in blanching, softening, and dissociation of liver tissue and provides complete digestion of the liver in 10–12 minutes.
4. Aseptically transfer the digested liver to a sterile 50-mL conical tube containing 15 mL cold L-15 Medium. Keep on ice and transport to cell culture facility.

   Note: The following procedures are to be carried out under sterile conditions in a laminar flow culture hood.

Purify hepatocytes

1. Disperse hepatocytes into single-cell suspension by pipetting with a large bore pipette. Centrifuge cell suspension at 50 × *g* for 5 minutes. Decant supernatant and resuspend cell pellet in 15 mL ice cold Hepatocyte Wash Medium. Pass cell suspension through a sterile 100-µm nylon mesh into a new sterile 50-mL conical tube on ice.
2. Wash hepatocytes-centrifuge cell suspension at 50 × *g* for 5 minutes, decant supernatant and resuspend cell pellet in 50 mL ice cold Hepatocyte Wash Medium. Repeat this step twice.
3. Purify hepatocytes by centrifugation on either 20% Nycodenz® or alternatively by Percoll® density gradient separation following product specific protocol.

4. Wash purified hepatocytes (see step 2 of this procedure) two times. Resuspend the cell pellet following the final wash in 25 mL Williams' Medium E, supplemented with 5 mL penicillin-streptomycin (100X) and determine viable cell density.

   Note: Typical results are~2.5 × 10^8 cells with 90–95% viability, as determined by trypan blue exclusion.

Culture Purified Hepatocytes

1. Prepare culture vessels ahead of time by coating with Rat Tail Collagen I (12.5 µg/cm^2) or Geltrex® (100 µg/cm^2) Reduced Growth Factor Basement Membrane Matrix according to product instructions. Wash flasks with Hepatocyte Wash Medium immediately prior to plating cells.
2. Plate ~1 × 10^7 purified hepatocytes per 150 cm^2 pre-coated culture flask in 25 mL attachment medium (Williams' Medium E, supplemented with 5 mL penicillin-streptomycin [100X] and either 30 µg/mL bovine fibronectin or 5% FBS) and incubate at 37°C in a humidified atmosphere of 5% CO_2 in air.
3. Decant and discard media, including any unattached cells, 2–3 hours after plating. Overlay attached cells with 25 mL HepatoZYME-SFM supplemented with 1.25 µg/cm^2 Rat Tail Collagen I to provide a sandwich matrix.
4. Replace culture medium with 25 mL complete HepatoZYME-SFM (without collagen) at 24 hours and every 48 hours thereafter. Hexobarbital (0.5–2.0 mM) or other inducing agents may be added to cultures 48 hours before harvesting the cells.

Analytical Procedures

1. Aspirate and discard culture media. Harvest cells by scraping into ice-cold 100 mM KPO_4 buffer (pH 7.4).
2. Sonicate and fractionate to yield a microsomal pellet that may be frozen at –70°C until use.
3. Homogenize microsomal pellet in a buffer containing protease inhibitors and use for protein (e.g., Western blot) and enzyme analysis.

Related products

Product	Catalog no.
Penicillin-Streptomycin, liquid	15140
L-Glutamine, 200 mM (100X), liquid	25030
GlutaMAX™-I, 200 mM (100X), liquid	35050
Certified FBS, Heat Inactivated, US	10082
Leibovitz's L-15	11415
William's Medium E (1X) without phenol red	A12176
Geltrex® Reduced Growth Factor Basement Membrane Matrix	A14132
Fibronectin, Bovine Plasma	33010
Collagen I, Rat Tail	A10483
HBSS, no calcium, no magnesium	14170
Trypan Blue Stain	15250
Countess® Automated Cell Counter	C10227

Explanation of symbols and warnings

The symbols present on the product label are explained below:

				
Temperature Limitation	Manufacturer	Batch code	Use By:	Catalog number
				
Caution, consult accompanying documents	Consult instructions for use	Keep away from light	Sterilized using aseptic processing techniques	

Limited Product Warranty

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