

Instruction Manual

■ Hepatocyte Growth Medium

Product	Size	Catalog Number
Hepatocyte Growth Medium (Ready-to-use)	500 ml	C-25010
Hepatocyte Growth Medium Kit	500 ml	C-25110
Hepatocyte Basal Medium	500 ml	C-25210
Hepatocyte Basal Medium, phenol red-free	500 ml	C-25215
Hepatocyte Growth Medium SupplementMix	for 500 ml	C-39642
Hepatocyte Growth Medium SupplementPack	for 500 ml	C-39640

■ Hepatocyte Maintenance Medium

Product	Size	Catalog Number
Hepatocyte Maintenance Medium (Ready-to-use)	500 ml	C-25020
Hepatocyte Maintenance Medium Kit	500 ml	C-25120
Hepatocyte Maintenance Medium SupplementMix	for 500 ml	C-39652
Hepatocyte Maintenance Medium SupplementPack	for 500 ml	C-39650

Recommended for

- Human Hepatocytes

Product Description

PromoCell Hepatocyte Media are serum-free media and have been developed for the *in vitro* cultivation of human hepatocytes. The medium is optimized for primary human cells, but can also be used for porcine hepatocytes.

PromoCell Hepatocyte Media are available as Medium (Ready-to-use) or as Medium Kit.

The Media (Ready-to-use) consist of a 500 ml bottle of Basal Medium and one vial of SupplementMix. The Medium Kit consists of a 500 ml bottle of Basal Medium and the SupplementPack (a set of individual vials with pre-measured supplements) allowing the user full control over the media formulation.

Adding the SupplementMix or the

SupplementPack to the Basal Medium results in the complete Growth Medium / Maintenance Medium.

Basal Medium (with or without phenol red) as well as SupplementMix and SupplementPacks can also be purchased separately.

Note: Under standard *in vitro* cell culture conditions, mature human hepatocytes usually do not survive for longer than 10–14 days and do not proliferate.

Final supplement concentrations (after addition to the medium)

	Hepatocyte Growth Medium	Hepatocyte Maintenance Medium
▪ Epidermal Growth Factor (recombinant human)	10 ng / ml	—
▪ Insulin (recombinant human)	5 µg / ml	6 ng / ml
▪ Hydrocortisone	0.5 µg / ml	—
▪ Transferrin, holo (human)	10 µg / ml	—
▪ Ascorbic Acid	250 µg / ml	—
▪ Bovine Serum Albumin-Fatty Acid Free (BSA-FAF)	3.75 mg / ml	—
▪ Dexamethasone	—	400 ng / ml

Supplementation Details

PromoCell Hepatocyte Growth Medium contains all the growth factors and supplements necessary for longterm cultivation of human hepatocytes, e.g. Epidermal Growth Factor, Insulin, and Transferrin (for details see the table above). If the media compounds of the Hepatocyte Growth Medium interfere with certain experimental setups, we recommend using the Hepatocyte Maintenance Medium. PromoCell Hepatocyte Maintenance Medium has been developed for short-term cultivation of hepatocytes and contains fewer supplements (for details see the table above). PromoCell Hepatocyte Media do not contain antibiotics or antimycotics and are formulated for use in an incubator with an atmosphere of 5% CO₂.

Note: For culturing Hepatocytes, Collagen type I coated culture vessels are necessary. Please make sure to have them before starting the protocol.

Preparation of the supplemented Medium for Use

Thaw the SupplementMix or SupplementPack at 15 to 25°C. Aseptically mix the supplement solutions by carefully pipetting up and down. Then, transfer the entire content of each supplement to the Basal Medium. Close the bottle and swirl gently until a homogenous mixture is formed.

Storage and Stability

Store the Basal Medium at 4 to 8°C in the dark and the SupplementMix or SupplementPack at -20°C immediately after arrival. Do not freeze the Basal Medium. If stored properly, the products are stable until the expiry date stated on the label. After adding the supplements to the Basal Medium, the shelf life of the complete medium is 6 weeks at 4 to 8°C. Do not freeze the complete medium. For use, pre-warm only an aliquot of the complete medium and keep the remaining medium refrigerated at 4 to 8°C.

Note: The SupplementMix and SupplementPack are delivered thawed and can be frozen after arrival without losing any activity.

Quality Control

All lots of PromoCell Hepatocyte Media are subjected to comprehensive quality control tests using primary human hepatocytes. Each lot is checked for adherence rate, typical morphology, and metabolism of the tested hepatocytes. Approved in-house lots of media are used as a reference.

In addition, all lots of media have been tested for the absence of microbial contaminants (fungi, bacteria, mycoplasma).

Intended Use

The products are for *in vitro* use only and not for diagnostic or therapeutic procedures. For safety precautions please see appropriate MSDS.

Note: Due to their low serum content or the absence of serum, PromoCell media are not suitable for trypsin neutralization (e.g. when splitting the cells). Instead we recommend to use our DetachKit (C-41200, C-41210, C-41220), which contains HEPES BSS, Trypsin/EDTA and Trypsin Neutralizing Solution.

If you require special media modifications, we offer a Custom Media Service starting at 10 bottles per order. Please ask for details.

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HepExtend™ Supplement (50X)

Description

HepExtend™ Supplement (50X) is an enriched supplement designed to enhance the culture lifespan of cryopreserved primary hepatocytes. Use the supplement in conjunction with Williams' Medium E (Cat. no. A12176) and the Hepatocyte Maintenance Supplement Pack (Cat. no. CM4000) following the recommended hepatocyte culture methods. When cultured in the presence of the HepExtend™ Supplement, primary human hepatocyte lots will live and be functional for 10 days or longer depending on the lot of cells and other assay conditions. HepExtend™ supplement does not contain growth factors, cytokines, fetal bovine serum, or pharmaceutical small molecules.

Product*	Catalog no.	Amount	Storage	Shelf life*
HepExtend™ Supplement (50X)	A27375-01	10 mL	-20°C to -5°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.

Safety information

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

Important information

Divide thawed HepExtend™ Supplement (50X) into aliquots and store in a non-frost-free freezer at -20°C to -5°C. Do not freeze-thaw HepExtend™ Supplement (50X) more than twice.

Prepare medium











1. Quickly thaw HepExtend™ Supplement (50X) in a 37°C waterbath (should thaw in under 10 minutes). Do not prolong exposure of the concentrated supplement to 37°C.
2. Prepare Hepatocyte Maintenance Medium by adding Hepatocyte Maintenance Supplement Pack (Cat. no. CM4000) to Williams E Medium (Cat. no. A12176). For further details on preparing hepatocyte media, refer to Gibco® [hepatocyte media supplement guide](#).
3. Add HepExtend™ Supplement (50X) to the Hepatocyte Maintenance Medium to a final dilution of 1X. For example, add 10 mL of HepExtend™ Supplement (50X) to 500 mL of Williams' E Medium for a 1X complete medium.
4. *Optional:* Filter complete medium using 0.22-µm filter units.
5. Once supplemented, the complete medium is stable for up to 3 weeks when stored at 2–8°C in the dark.
6. Warm complete medium in a 37°C waterbath prior to daily feeding of cells. We recommend aliquotting the medium and warming only what is needed for the daily feed, keeping the main batch of medium at 2–8°C.
7. Refer to Gibco® [Thawing and Plating Cryopreserved Hepatocytes](#) protocol for detailed information on plating and culturing primary cryopreserved hepatocytes.

Note: HepExtend™ Supplement (50X) contains 0.12375 g/mL bovine serum albumin (BSA). Hepatocyte Maintenance Supplement (Cat. no. CM4000) contains 0.125 g/mL BSA, prior to dilution. The final complete HepExtend™ Hepatocyte Maintenance Medium will contain 0.003725 g/mL BSA.

Related products

Product	Cat. no.
Williams' Medium E (1X) without L-glutamine, without phenol red	A12176
Primary Hepatocyte Maintenance Supplements	CM4000
Primary Hepatocyte Thawing and Plating Supplements	CM3000
Geltrex® LDEV-Free Reduced Growth Factor Basement Membrane Matrix	A14132
Collagen I, Coated Plate	A11428
Hepatocyte Thaw Medium	CM7500
Human Plateable Hepatocytes, Transporter Qualified	HMCPST
Human Plateable Hepatocytes, Induction Qualified	HMCPIS
Human Plateable Hepatocytes, Metabolism Qualified	HMCPMS

Explanation of symbols and warnings

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

Limited product warranty

Life Technologies Corporation and/or its affiliate(s) warrant their products as set forth in the Life Technologies' General Terms and Conditions of Sale found on Life Technologies' website at www.lifetechnologies.com/termsandconditions.

If you have any questions, please contact Life Technologies at www.lifetechnologies.com/support.

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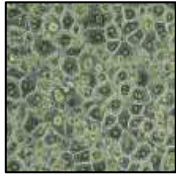
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S C I E N T I F I C



Clonetics™ Human Hepatocyte Cell Systems

h NHEPS™ Cells– Instructions for Use

Introduction

Clonetics™ Hepatocyte Cell Culture System contains Normal Human Primary Hepatocytes (h NHEPS™ Cells) isolated from single donors. Each cryopreserved ampoule will yield $\geq 3 \times 10^6$ viable human hepatocytes. The recommended seeding density for adherent hepatocytes is 150,000 cells/cm² on human or rat-tail type 1 collagen coated vessels or 200,000 cells/cm² on Matrigel® coated vessels. For optimal attachment, Corning Cellbind® plasticware (plates or flasks) coated with human or rat-tail type 1 collagen or Matrigel® is strongly recommended. For optimal performance, pre-coated culture vessels are not recommended. It is recommended to add 2% FBS to the Hepatocyte Culture Medium or Hepatocyte Maintenance Medium for initial plating and change to serum free medium at the first media change (after three hours). These cells do not proliferate.

Unpacking and Storage Instructions

1. Check all containers for leakage or breakage.
2. For cryopreserved cells: Remove cryovials from the dry ice packaging and immediately place into liquid nitrogen storage. Alternatively, thaw and use the cells immediately. If no dry ice remains, please contact Customer Service.
3. BulletKit™ Medium instructions: store basal medium (HBM™ or HMM™) at 2°-8°C and SingleQuots™ Kit (HCM™ or HMM™) at $\leq 20^\circ\text{C}$ in a freezer that is not self-defrosting. Once thawed, SingleQuots™ Kit should be stored at 2°-8°C and added to basal medium

within 72 hours. After SingleQuots™ Kit is added to basal medium, use within 1 month. Do not re-freeze.

Using media or reagents other than what is recommended will void the cell warranty. Please contact Scientific Support if you need help selecting media and/or reagents.

Preparation of Media

1. Decontaminate external surfaces of all vials and the medium bottle with ethanol or isopropanol.
2. To formulate Hepatocyte Culture Medium (HCM™ Medium), transfer the contents of the HCM™ SingleQuots™ Kit [Catalog No. CC-4182 containing Ascorbic Acid, Bovine Serum Albumin – Fatty Acid Free (BSA-FAF), Hydrocortisone, human Epidermal Growth Factor (hEGF), Transferrin, Insulin, and Gentamicin/Amphotericin-B (GA)] to HBM™ Basal Medium with a pipette, and rinse each vial with medium.
3. To formulate Hepatocyte Maintenance Medium (HMM™ Medium), transfer the contents of the HMM™ SingleQuots™ Kit [Catalog No. CC-4192 containing Dexamethasone, Insulin, and Gentamicin/Amphotericin-B (GA)], to HMM™ Basal Medium with a pipette, and rinse each vial with medium (Catalog No. CC-3197).
4. When preparing these BulletKit™ Media, it may not be possible to recover the entire volume listed for each vial. Small losses (up to 10%) should not affect the cell growth characteristics of the supplemented medium.

5. Transfer the label provided with each kit to the basal medium bottle(s) being supplemented (avoid covering the basal medium lot # and expiration date). Use it to record the date and amount of each supplement added.

NOTE: If there is concern that sterility was compromised during the supplementation process, the entire newly prepared growth medium may be re-filtered with a 0.2 µm filter to assure sterility. Routine re-filtration is not recommended.

Thawing of Adherent Cells / Initiation of Culture Process

NOTE: For optimal attachment, Corning Cellbind® plasticware (plates or flasks) coated with human or rat-tail type 1 collagen or Matrigel® is strongly recommended. For optimal performance, pre-coated culture vessels are not recommended. It is recommended to add 2% FBS (not included) to the Hepatocyte Culture Medium or Hepatocyte Maintenance Medium for initial plating and change to serum free medium at the first media change (after three hours).

1. The recommended seeding density for adherent hepatocytes is 150,000 cells/cm² on vessels coated with 60 µg/cm² human or rat-tail type 1 collagen coated or 200,000 cells/cm² on Matrigel® coated vessels.
2. Wipe cryovial with ethanol or isopropanol before opening. In a sterile field, briefly twist the cap a quarter turn to relieve pressure, and then retighten. Quickly thaw the cryovial in a 37°C water bath being careful not to submerge the entire vial. Watch your cryovial closely; when the last sliver of ice melts, remove it. Do not submerge it completely. Thawing the cells for longer than 2 minutes results in less than optimal results.
3. Resuspend the cells in the cryovial and using a micropipette, slowly transfer the cell suspension to a conical tube containing 20 ml of cold HCM™ Medium or HMM™ Medium.
4. Centrifuge the cell suspension cells at 50 x g in a refrigerated centrifuge (2°-8°C) for three minutes to pellet the cells.
5. Gently aspirate most of the supernatant, except for 100-200 µl, without disturbing the cell pellet.
6. Flick the cryovial with your finger to loosen the pellet.
7. Dilute the cells to a final volume of 10 ml in cold HCM™ Medium containing 2% FBS (not included) or HMM™ Medium containing 2% FBS (not included).
8. Determine cell count and viability using a hemacytometer and Trypan Blue. Make a note of your cell yield for later use.

9. Adjust cell density to the desired concentration using HCM™ Medium containing 2% FBS (not included) or HMM™ Medium containing 2% FBS (not included) for plating. For human or rat-tail type 1 collagen coated vessels, the appropriate density is 300,000 viable cells/ml. For Matrigel® coated vessels, the appropriate density is 400,000 viable cells/ml.
10. Dispense cells into the culture vessels set up earlier using 0.5 ml of cell suspension per cm² of the plating surface. Gently rock the culture vessel to evenly distribute the cells and place the vessels in a 5% CO₂, 37°C incubator.
11. After three hours, replace the media with fresh, pre-warmed HCM™ Medium (without FBS) or HMM™ Medium (without FBS) and return vessels to the incubator

Maintenance of Adherent Cells

NOTE: Adherent hepatocytes will survive for up to seven days in Hepatocyte Culture Medium (HCM™ Medium), a serum-free medium for maintenance of cultures prior to and during experimental procedures in a metabolically active state. Adherent hepatocytes will survive for up to two days in Hepatocyte Maintenance Medium (HMM™ Medium), a serum-free medium for maintenance of cultures prior to and during experimental procedures in a more basal, less-metabolically active state.

1. Change the medium (without FBS) three hours after seeding and **every day** thereafter.
2. Warm an appropriate amount of medium to 37°C in a sterile container. Remove the medium and replace it with the warmed, fresh medium and return the flask to the incubator.
3. Avoid repeated warming and cooling of the medium. If the entire contents are not needed for a single procedure, transfer and warm only the required volume to a sterile secondary container.

Thawing of Suspension Cells / Initiation of Culture Process

1. Wipe cryovial with ethanol or isopropanol before opening. In a sterile field, briefly twist the cap a quarter turn to relieve pressure, and then retighten. Quickly thaw the cryovial in a 37°C water bath being careful not to submerge the entire vial. Watch your cryovial closely; when the last sliver of ice melts, remove it. Do not submerge it completely. Thawing the cells for longer than 2 minutes results in less than optimal results.
2. Resuspend the cells in the cryovial and using a micropipette, slowly transfer the cell suspension

to a conical tube containing 20 ml of cold HMM™ Medium.

3. Centrifuge the cell suspension cells at 50 x g in a refrigerated centrifuge (2°-8°C) for three minutes to pellet the cells.
4. Gently aspirate most of the supernatant, except for 100-200 µl, without disturbing the cell pellet.
5. Flick the cryovial with your finger to loosen the pellet.
6. Dilute the cells to a final volume of 10 ml in cold HMM™ Medium.
7. Determine cell count and viability using a hemacytometer and Trypan Blue. Make a note of your cell yield for later use.
8. Adjust cell density to the desired concentration using HMM™ Medium.
9. Dispense cells into the desired culture vessels and place the vessels in a 5% CO₂, 37°C incubator.

Maintenance of Suspension Cells

NOTE: Suspension hepatocytes will survive for up to twelve hours in Hepatocyte Maintenance Medium (HMM™ Medium), a serum-free medium for maintenance of cultures prior to and during experimental procedures in a more basal, less-metabolically active state.

1. Change the medium as necessary.
2. Warm an appropriate amount of medium to 37°C in a sterile container. Remove the medium and replace it with the warmed, fresh medium and return the flask to the incubator.
3. Avoid repeated warming and cooling of the medium. If the entire contents are not needed for a single procedure, transfer and warm only the required volume to a sterile secondary container.

Quality Control

All cells are performance assayed and test negative for HIV-1, Hepatitis-B, and Hepatitis-C. Cultures should always be considered infectious. The end user must take the proper precautions when using cells derived from human tissue. Cell viability and cell number are measured after recovery from cryopreservation for all cells. Cell morphology and attachment are measured after recovery from cryopreservation for adherent cells only. Clonetics™ Media are formulated for optimal culturing or maintenance of specific types of normal human cells. Certificates of Analysis (COA) for each cell

strain are shipped with each order. COAs for all other products are available upon request.

Ordering Information

Cryopreserved Hepatocytes (Single Donor):

Cat. No.	Product	Description
CC-2591	h NHEPS™ Adherent Cells	3-6 million adherent cells
CC-2591S	h NHEPS™ Suspension Cells	3-6 million suspension cells

Hepatocyte Culture Media (Sold Separately):

Cat. No.	Product	Description
CC-3198	HCM™ BulletKit™ Medium	500 ml HBM™ Basal Medium, phenol red free plus CC-4182 SingleQuots™ Kit to formulate HCM™ Medium (culture medium)
CC-3199	HBM™ Basal Medium	Hepatocyte basal medium (500 ml), phenol red free
CC-4182	HCM™ SingleQuots™ Kit	Formulates 500 ml of HBM™ Basal Medium to HCM™ Culture Medium; contains Ascorbic Acid, 0.5 ml; BSA-FAF, 10 ml; Hydrocortisone, 0.5 ml; human hEGF, 0.5 ml; Transferrin, 0.5 ml; Insulin, 0.5 ml; GA, 0.5 ml

Hepatocyte Maintenance Media (Sold Separately):

Cat. No.	Product	Description
CC-3197	HMM™ Basal Medium	Hepatocyte Maintenance Media basal medium (500 ml), phenol red free
CC-4192	HMM™ SingleQuots™ Kit	Formulates 500 ml of HMM™ Basal Medium to HMM™ Maintenance Medium; contains Dexamethasone, 0.5 ml; Insulin, 0.5 ml; GA, 0.5 ml

Product Warranty

Cultures have a finite lifespan *in vitro*.

Lonza guarantees the performance of its cells only if Clonetics™ Media and Reagents are used exclusively, and the recommend protocols are followed. The performance of cells is not guaranteed if any modifications are made to the complete cell system.

When placing an order or for Scientific Support, please refer to the product numbers and

descriptions listed above. For a complete listing of all Clonetics™ Products, refer to the Lonza website or the current Lonza catalog. To obtain a catalog, additional information or want to speak with Scientific Support, you may contact Lonza by web, e-mail, telephone, fax or mail (See page 1 for details).

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WARNING: CLONETICS™ AND POIETICS™ PRODUCTS CONTAIN HUMAN SOURCE MATERIAL, TREAT AS POTENTIALLY INFECTIOUS. Each donor is tested and found non-reactive by an FDA-approved method for the presence of HIV-1, 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. Testing cannot offer complete assurance that HIV-1, hepatitis B virus, and hepatitis C virus are absent. All human-sourced products should be handled at the biological safety level 2 to minimize exposure of potentially infectious products, as recommended in the CDC-NIH manual, [Biosafety in Microbiological and Biomedical Laboratories](#), 5th ed. If you require further information, please contact your site safety officer or Scientific Support.

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