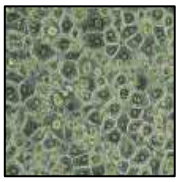
Lonza

Lonza Walkersville, Inc.
Walkersville, MD 21793-0127 USA
U.S. Scientific Support: 800 521 0390
scientific.support@lonza.com
EU/ROW Scientific Support: +32 87 321 611
scientific.support.eu@lonza.com
Document # AA-1014-14 08/12
www.lonza.com



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 ≥ 3 x 10^6 viable human hepatocytes. The recommended seeding density for adherent hepatocytes is 150,000 cells/cm^2 on human or rat-tail type 1 collagen coated vessels or 200,000 cells/cm^2 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 Maintenence 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 ≤20°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 Maintenence Medium (HMM™ Medium), transfer the contents of the HHM™ 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 Maintenence 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/cm^2 on vessels coated with 60 µg/cm^2 human or rat-tail type 1 collagen coated or 200,000 cells/cm^2 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℃ 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℃) 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 cm^2 of the plating surface. Gently rock the culture vessel to evenly distribute the cells and place the vessels in a 5% CO_2, 37℃ 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 Maintenence 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℃ 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℃ 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% CO_2, 37°C incubator.

Maintenance of Suspension Cells

NOTE: Suspension hepatocytes will survive for up to twelve hours in Hepatocyte Maintenence 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).

THESE PRODUCTS ARE FOR RESEARCH USE ONLY. Not approved for human or veterinary use, for application to humans or animals, or for use in clinical or *in vitro* procedures.

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-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. 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.