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Product Information

HepaRG™ PXR Knockout Cells
HepaRG™ Cryopreserved Vials

Catalog Number **MTOX1011**
Storage Temperature –130 °C or below
in liquid nitrogen vapor phase

TECHNICAL BULLETIN

Product Description

CompoZr® zinc finger nuclease (ZFN) technology is a fast and reliable way to manipulate the genome in a targeted fashion. ZFNs are naturally occurring proteins that can be engineered to bind DNA at a sequence-specific location and create a double strand break (sigma.com/zfn). The cell's natural machinery repairs the break in one of two ways: non-homologous end joining or homologous recombination. The non-homologous end joining pathway typically produces small modifications (indels) at the targeted locus that may result in a functional knockout. Single cell clones are then isolated, tested for the desired modification, and expanded to establish stable cell lines.

HepaRG™ is a human hepatoma cell line isolated in 2002 from a liver tumor of a female patient suffering from hepatocarcinoma and hepatitis C infection. The cells possess a pseudodiploid karyotype and have been characterized as an oval ductular bipotent hepatic cell line as they have the ability to differentiate into both hepatocyte and cholangiocyte-like lineages in the presence of DMSO.

HepaRG cells express the major xenobiotic sensors, drug transporters, and phase I and II drug metabolizing enzymes as well as key hepatic transcription factors involved in stress response pathways. Several recent publications show the cells are suitable for studies on drug metabolism, CYP induction, metabolism-mediated toxicity, and genotoxicity. Because of these unique properties HepaRG cells were selected as the background cell line to use for the development of hepatocyte-specific knockout cells.

This product consists of ZFN engineered HepaRG PXR Knockout Cells. They are intended for use with modified HepaRG Control Cells (Catalog Number MTOX1010) for a wide variety of liver cell based assays.

Species-specific PCR Evaluation:
The cells were confirmed to be of human origin and no mammalian interspecies contamination was detected.

PCR Evaluation for *Mycoplasma sp*. contamination and human infectious agents, including Hepatitis C virus:
Negative

Components

This product contains 10 million cryopreserved modified HepaRG cells. These cells were modified with the use of transfected mRNA encoding ZFNs targeting a specific site.

Neither media nor supplements are supplied with the vials. These must be obtained prior to receiving the vials.

Precautions and Disclaimer

This product is for R&D use only, not for drug, household, or other uses. Please consult the Safety Data Sheet for information regarding hazards and safe handling practices.

Storage/Stability

Upon receiving a shipment of frozen cells it is important the end user gives the shipment attention without delay. To ensure the highest level of viability, thaw the vial and initiate the culture as soon as possible upon receipt. If upon arrival, continued storage of the frozen culture is necessary, it should be stored in liquid nitrogen vapor phase and not at –70 °C. Storage at –70 °C will result in loss of viability.

Procedures

Protocol for Thawing and Seeding 24 Well Plates

Note: One cryovial of HepaRG cells contains enough cells to seed one plate.

Reagents and Equipment Required but Not Provided for Thawing and Seeding

Note: Neither media nor supplements are supplied with the vials. These must be obtained prior to receiving the vials.

- HepaRG Thawing and Plating Medium Supplement (Biopredic International ADD671C)
  Note: Once prepared, the medium can be stored at 2–8 °C for up to 1 month.
- Williams' Medium E (Catalog Number W1878)
- Penicillin-Streptomycin (Catalog Number P4333)
- 200 mM Ala-Gln solution (Catalog Number G8541)
- Corning® BioCoat™ Collagen I, 24 well plate (Corning 356408)
- BSL-2 hood
- Cell culture incubator

Preparation of Thawing and Plating Medium

Add 1 mL of Penicillin-Streptomycin solution, 1 mL of 200 mM Ala-Gln solution, and entire HepaRG Thawing and Plating Medium Supplement to 100 mL of Williams' Medium E in a sterile container (do not filter).

Step 1: Thawing Cells and Seeding Plates

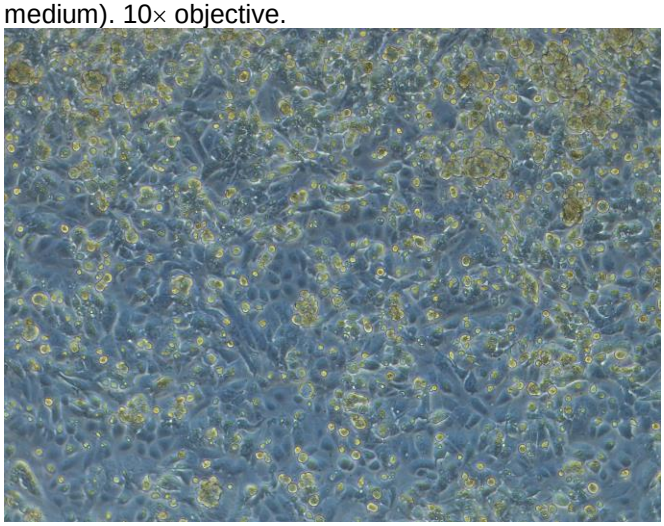
1. Pre-warm HepaRG Thawing and Plating Medium in 37 °C water bath.
2. Pipette pre-warmed, Thawing and Plating Medium (<23 mL per HepaRG cryovial to be used per plate) into a sterile container to be used for mixing and plating cells.
3. Wet an absorbent paper with 70% ethyl alcohol.
4. Remove cryovial from liquid nitrogen. Under a laminar flow hood, briefly twist the cap a quarter turn to relieve the internal pressure and then close again.
5. Quickly transfer the cryovial to a 37 °C water bath. While holding the tip of the vial, gently agitate for 1–2 minutes, being careful not to allow water to penetrate the cap.
   Note: **Do not submerge cryovial completely**.
6. Watch the cryovial closely. When just a small crystal of ice remains, remove it from the water bath.
7. Wipe the outside of the vial with absorbent paper moistened with 70% ethyl alcohol and place it under laminar flow hood.
8. Aseptically transfer the cell suspension to the sterile container with pre-warmed Thawing and Plating Medium.
9. Mix cells thoroughly and aliquot 1 mL per well in a 24 well plate.
10. Gently shake the plate to evenly distribute cells in wells. Place plate in incubator at 37 °C, 5% CO_2, and saturating humidity.

Step 2: 48 hours Post-Thawing and Seeding

Aspirate the old medium and replenish with 1 mL of Thawing and Plating Medium per well.

Figure 1.

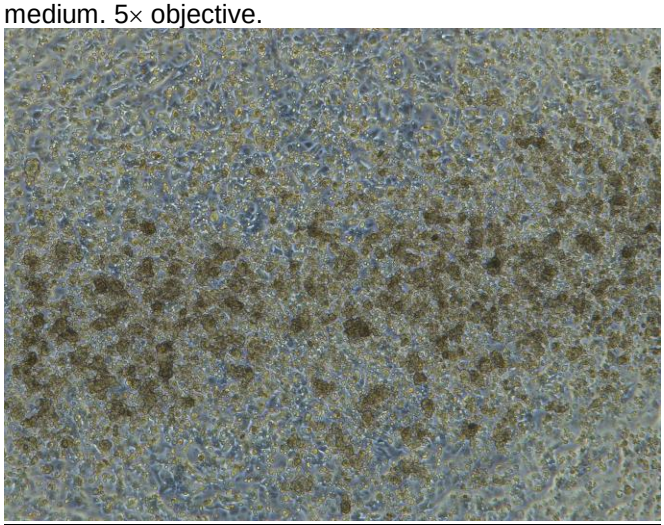
Cells at 48 hours post-thawing (thawing and plating medium). 10× objective.



Step 3: Confluency Determination

Figure 2.

Confluent cells - ready to switch to differentiation medium. 5× objective.



1. 24 hours after changing medium, observe cells under microscope, and check for confluency (see Figure 2).

2. If cells are not confluent, remove medium and replace with 1 mL of Thawing and Plating Medium twice a week until confluence. Refer to CofA to confirm cell morphology at confluency for individual cell lines. Continue to observe cells daily, and once confluent, replace medium with 1 mL of maintenance medium.
   or
   If confluent, remove medium and replace with 1 mL of maintenance medium.
3. Cells should be differentiated for a minimum of 14 days, exchanging medium twice weekly before beginning these assays (see Figure 3).
4. Proceed with culture conditions for either:

   Basal Metabolism Assay
   or
   Induction Assay
   or
   Sandwich Culture Model Assay

Figure 3.
Day 15 of differentiation - ready for metabolism, induction, and sandwich culture assays. 10× objective.

Basal Metabolism Assay
Reagents Required but Not Provided for Basal Metabolism Assay
Note: Neither media nor supplements are supplied with the vials. These must be obtained prior to receiving the vials.

- HepaRG Maintenance and Metabolism Medium Supplement (Biopredic International ADD621C)
- Williams' Medium E (Catalog Number W1878)
- Penicillin-Streptomycin (Catalog Number P4333)
- 200 mM Ala-Gln solution (Catalog Number G8541)
- Corning BioCoat Collagen I, 24 well plate (Corning 356408)

Preparation of Maintenance and Metabolism Medium
Add 1 mL of Penicillin-Streptomycin solution, 1 mL of 200 mM Ala-Gln solution, and entire HepaRG Maintenance and Metabolism Supplement to 100 mL of Williams' Medium E in a sterile container (do not filter).

Basal Metabolism Assay Protocol
1. Culture confluent cells in Maintenance and Metabolism Medium for a minimum of 14 days, exchanging medium twice weekly.
2. Remove maintenance medium and wash cells with unsupplemented Williams' Medium E.
3. Perform basal assay in unsupplemented Williams' Medium E according to established protocols.

Induction Assay
Reagents Required but Not Provided for Induction Assay
Note: Neither media nor supplements are supplied with the vials. These must be obtained prior to receiving the vials.

- HepaRG Maintenance and Metabolism Medium Supplement (Biopredic International ADD621C)
- HepaRG Induction Medium Supplement (Biopredic International ADD641C)
- HepaRG Serum Free Induction Medium Supplement (Biopredic International ADD651C)
- Williams' Medium E (Catalog Number W1878)
- Penicillin-Streptomycin (Catalog Number P4333)
- 200 mM Ala-Gln solution (Catalog Number G8541)

Preparation of Maintenance and Metabolism, Induction, or Serum Free Induction Media
Add 1 mL of Penicillin-Streptomycin solution, 1 mL of 200 mM Ala-Gln solution, and the entire specific medium supplement to 100 mL Williams' Medium E in a sterile container (do not filter).

Induction Protocol
1. Culture confluent cells in Maintenance and Metabolism Medium for a minimum of 14 days, exchanging medium twice weekly.
2. Aspirate the old medium and replace with 1 mL of Induction Medium per well.
3. Return plate to incubator for an additional 48 hours.
4. Remove Induction Medium from each well.
5. Add 1 mL of test article in Serum Free Induction Medium to each well.
6. Refresh with test article in Serum Free Induction Medium daily for 2–3 days.
7. Remove Serum Free Induction Medium containing test article.
8. Wash wells with unsupplemented Williams' Medium E.

9. Follow established protocols for measuring induction of CYP activity or mRNA.
   Note: For substrates and inhibitors, visit sigma.com/admetox

Sandwich Culture Model Assay

Reagents Required but Not Provided for Sandwich Culture Model

Note: Neither media nor supplements are supplied with the vials. These must be obtained prior to receiving the vials.

- HepaRG Thawing and Plating Medium Supplement (Biopredic International ADD671C)
- HepaRG Maintenance and Metabolism Medium Supplement (Biopredic International ADD621C)
- Williams' Medium E (Catalog Number W1878)
- Penicillin-Streptomycin (Catalog Number P4333)
- 200 mM Ala-Gln solution (Catalog Number G8541)
- Corning BioCoat Collagen I, 24 well plate (Corning 356408)
- Corning Matrigel® Basement Membrane Matrix (Corning 356237)

Preparation of Thawing and Plating, or Maintenance and Metabolism Media

Add 1 mL of Penicillin-Streptomycin solution, 1 mL of 200 mM Ala-Gln solution, and the entire specific medium supplement to 100 mL Williams' Medium E in a sterile container (do not filter).

Sandwich Protocol

1. Culture confluent cells in Maintenance and Metabolism Medium for a minimum of 12 days, exchanging medium twice weekly.
2. Aspirate the old medium and wash cells once with ice-cold Maintenance and Metabolism Medium.
3. Add Matrigel to a final concentration of 0.25 mg/mL in ice-cold Maintenance and Metabolism Medium.
4. Overlay the cells with 500 µL of the Matrigel mixture per well.
5. Culture cells for 3 days in Matrigel with one medium change.
6. Perform assay according to established protocols.

JB,DA,KA,MDM,AA,MAM,TL 08/18-1

These products are covered by the License Agreement as described in Exhibit 1 and 2.

EXHIBIT 1

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7. Products covered by a "Consumable Vial" License can only be incorporated in an end-use assay, seeded directly on the final assay plate (including, but not limited to 6-well, 12-well, 24-well, 48-well, or 96-well plates). All Products that are not utilized in an end-use assay plates shall be immediately destroyed.

8. Products delivered in a Petaka cell culture device can only be incorporated in an end-use assay for up to two (2) transwell plates (including, but not limited to 24-well or 96-well plates). All Products that are not utilized in an end-use assay for up to two (2) transwell plates shall be immediately destroyed.

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EXHIBIT 2

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These cells are derived from HepaRG™ cells and are protected by patents; by opening this package, you agree to abide the terms of this Limited Use License as follows. You will consider the cells as a disposable product to be destroyed upon conclusion of a study or experiment; will use them only for in-vitro experiments and only at the facility of their receipt; will not propagate, amplify, reproduce, clone, or make any other use of them after a study is concluded; will not produce or manufacture commercial products or cDNA libraries or cell stock from the cells; will not use them for any study of a duration exceeding 20 days; and you will not license, resell or transfer them to anyone outside your organization for any reason. If you are unwilling to accept the terms of this LIMITED USE LICENSE, do not use the cells and return them to SA for credit. Violators of this LIMITED USE LICENSE will be prosecuted to the fullest extent of the law.

HepaRG™ WildType LIMITED USE LICENSE

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