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Product Information

CompoZr® ADME/Tox Cell Lines
OATP1B1 Knockout HepaRG™ Cells

Catalog Number **MTOX1015**
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 (www.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.[1] 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 biliary and hepatocyte lineages in the presence of DMSO.[2]

HepaRG cells express the major xenobiotic sensors (PXR, CAR, and AhR), drug transporters, and phase I and II drug metabolizing enzymes as well as key hepatic transcription factors involved in stress response pathways. In particular, HepaRG cells are the most metabolically active human hepatocyte cell line developed to date, especially relative to CYP3A4. Several recent publications suggest the cells are suitable for studies on drug metabolism, CYP induction, metabolism-mediated toxicity, and genotoxicity.[3-6] 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 OATP1B1 Knockout HepaRG cells. They are intended for use with 5F Clone 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:
Negative

Component

This product is a cryovial containing at least 10 million OATP1B1 Knockout HepaRG cells.

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.

Procedures

A. Protocol for Thawing and Seeding 24 Well Plates

Note: One cryovial of OATP1B1 Knockout 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.

- Recovery Medium Supplement (Catalog Number MTOXHRSUP), 72 mL – The medium can be stored at 2–8 °C for up to 1 month.
- Williams' E Medium (Catalog Number W1878)
- Penicillin-Streptomycin (Catalog Number P4333)
- GlutaMAX™ Supplement (Life Technologies 35050-061)
- Corning 24 well plate (Catalog Number CLS3527) or Corning® BioCoat™ Collagen I, 24 well plate (Corning 356408)
- BSL-2 hood
- Cell culture incubator

Preparation of Recovery Medium

Add 5 mL of Penicillin-Streptomycin solution, 5 mL of GlutaMAX, and entire Recovery Supplement to 500 mL of Williams' Medium E (do not filter).

Day 0

1. Pre-warm Recovery Medium in 37 °C water bath.
2. Pipette ~23 mL per HepaRG cryovial to be used per plate of pre-warmed, Recovery Medium into a sterile container to be used for mixing and plating cells.
3. Prepare 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 70% ethyl alcohol absorbent paper and place it under laminar flow hood.
8. Aseptically transfer the cell suspension to the sterile container with pre-warmed Recovery 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.

Day 2

1. Aspirate the old medium and replenish with 1 mL of Recovery Medium per well.
2. Proceed with culture conditions for either:
   Sandwich Culture Model (Procedure B)
   or
   Induction Assay (Procedure C)

B. Protocol for Sandwich Culture Model

Reagents and Equipment 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.

- Recovery Medium Supplement (Catalog Number MTOXHRSUP), 72 mL – The medium can be stored at 2–8 °C for up to 1 month.
- Williams' E Medium (Catalog Number W1878)
- Penicillin-Streptomycin (Catalog Number P4333)
- GlutaMAX Supplement (Life Technologies 35050-061)
- Corning Matrigel® Basement Membrane Matrix (Corning 356237)
- BSL-2 hood
- Cell culture incubator

Preparation of Recovery Medium

Add 5 mL of Penicillin-Streptomycin solution, 5 mL of GlutaMAX, and entire Recovery Supplement to 500 mL of Williams' Medium E (do not filter).

Day 4

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

Day 7

1. Aspirate the old medium and wash cells once with ice-cold Recovery Medium.
2. Add Matrigel to a final concentration of 0.25 mg/mL in ice-cold Recovery Medium.
3. Overlay the cells with 500 µL of the Matrigel mixture per well.
4. Change medium every other day until assay day, replenishing with 1 mL of Recovery Medium per well.

Day 10

Assay Day – Perform assay according to established protocols.

C. Protocol for Induction Assay

Reagents and Equipment 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.

- Maintenance Medium Supplement (Catalog Number MTOXHMSUP), 72 ml – The medium can be stored at 2–8 °C for up to 1 month.
- Pre-induction Medium Supplement (Catalog Number MTOXHPSUP), 72 ml – The medium can be stored at 2–8 °C for up to 1 month.
- Serum Free Induction Medium Supplement (Catalog Number MTOXHSFISUP), 4 ml – The medium can be stored at 2–8 °C for up to 1 month.
- Williams' E Medium (Catalog Number W1878)
- Penicillin-Streptomycin (Catalog Number P4333)
- GlutaMAX Supplement (Life Technologies 35050-061)
- BSL-2 hood
- Cell culture incubator

Preparation of Maintenance, Pre-induction, or Serum Free Induction Media

Add 5 mL of Penicillin-Streptomycin solution, 5 mL of GlutaMAX, and entire specific Supplement to 500 mL Williams' Medium E (do not filter).

Day 4

1. Aspirate the old medium and replace with 1 mL of Maintenance Medium per well.
2. Repeat on the following Monday, Wednesday, and Friday for two weeks.

Day 18

1. Remove Maintenance Medium from each well.
2. Add 1 mL of fresh Pre-induction Medium into each well. Put the plates back into incubator to incubate over weekend.

Day 21

1. Remove Pre-induction Medium from each well.
2. Add 1 mL of test article in Serum-Free Induction Medium to each well.
3. Refresh with test article in Serum-Free Induction Medium daily.

Day 24

1. Remove Serum-Free Induction Medium containing test article.
2. Wash wells with HBSS or PBS.
3. Add probe substrate in unsupplemented Williams' Medium E.

References

1. Gripon, P. *et al*., (2002) Infection of a human hepatoma cell line by hepatitis B virus. Proc. Natl. Acad. Sci. *USA*., **99**, 15655-15660.
2. Parent *et al*., (2004) Origin and characterization of a human bipotent liver progenitor cell line. Gastroenterology, **126**, 1147-1156.
3. Andersson, T.B. *et al*., (2012) The HepaRG cell line: a unique *in vitro* tool for understanding drug metabolism and toxicology in human. Expert Opin. Drug Metab. Toxicol., **8**, 909-920.
4. Kanebratt, K.P., and Andersson, T.B., (2008) HepaRG cells as an *in vitro* model for evaluation of cytochrome p450 induction in humans. Drug Metab. Dispos., **36**, 137-145.
5. McGill, M.R. *et al*., (2011) HepaRG cells: a human model to study mechanisms of acetaminophen hepatotoxicity. Hepatology, **53**, 974-982.
6. Le Hegarat, L. *et al*., (2010) Assessment of the genotoxic potential of indirect chemical mutagens in HepaRG cells by the comet and the cytokinesis-block micronucleus assays. Mutagenesis, **25**, 555-560.

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(a) Not expand, propagate, seed, bank, cryopreserve or store the product, its components, or any materials made through the use of this Product, outside of the original shipping device, including but not limited to vial, transwell plate and Petaka cell culture device. The Product can only be transferred and/or incorporated in an end-use assay. All Products that are not utilized in an end-use assay shall be immediately destroyed.

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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

HepaRG™ LIMITED USE LICENSE

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.