3050 Spruce Street, St. Louis, MO 63103 USA
Tel: (800) 521-8956 (314) 771-5765 Fax: (800) 325-5052 (314) 771-5757
email: techservice@sial.com sigma-aldrich.com

Product Information

HepaRG™ Assay Ready Plates containing HepaRG CAR Knockout Cells

Catalog Number **MTOX1012P96**

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 HepaRG CAR Knockout 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

Components

This product contains differentiated HepaRG cells seeded on 96 well plates.

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

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 plated HepaRG Induction Assay in 96-well plates

Reagents and Equipment Required but Not Provided.
Note: Neither media nor supplements are supplied with the plates. These must be obtained prior to receiving the plates.

- Recovery Medium Supplement (Catalog Number MTOXHRSUP), 72 ml – The medium can be stored at 2–8 °C for up to 1 month.
- 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)
- BSL-2 hood
- Cell culture incubator

Media Preparation

1. 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).

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

3. Pre-induction Medium – Add 5 ml of Penicillin-Streptomycin solution, 5 ml of GlutaMAX, and entire Pre-induction Supplement to 500 ml of Williams' Medium E (do not filter).

4. Serum Free Induction Medium – Add 5 ml of Penicillin-Streptomycin solution, 5 ml of GlutaMAX, and entire Serum Free Induction Supplement to 500 ml of Williams' Medium E (do not filter).

Day 1 (plates received on Thursday)

1. Remove the old medium from each well.
2. Add 200 µl of fresh Recovery Medium into each well. Put the plates back into incubator to allow ≥3 days of recovery (over the weekend).

Day 5 (Monday)

1. Aspirate the old medium and replace with Maintenance Medium.
2. Repeat on Day 7 (Wednesday).

Day 9 (Friday)

1. Remove Maintenance Medium from each well.
2. Add 200 µl of fresh Pre-induction Medium into each well. Put the plates back into incubator to incubate over the weekend.

Day 12 (Monday)

1. Remove Pre-induction Medium from each well.
2. Add 200–400 µl of test article in Serum Free Induction Medium to each well.
3. Refresh with test article in Serum Free Induction Medium on Days 13 and 14 (Tuesday and Wednesday, respectively).

Day 15 (Thursday)

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

B. Protocol for Sandwich Culture Model (Transporter Assays)

Reagents and Equipment Required but Not Provided for Sandwich Culture Model.
Note: Neither media nor supplements are supplied with the plates. These must be obtained prior to receiving the plates.

- Recovery Medium Supplement (Catalog Number MTOXHRSUP), 72 ml – 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)
- GlutaMAX™ Supplement (Life Technologies 35050-061)
- Corning® Matrigel® Basement Membrane Matrix (Corning 356237)
- BSL-2 hood
- Cell culture incubator

Recovery Medium Preparation
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 1

Aspirate the old medium and replenish with 200 µl of Recovery Medium per well.

Day 5

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 200 µl of the Matrigel mixture per well.
4. Change medium every other day until assay day, replenishing with 200 µl of Recovery Medium per well.

Day 10 (Perform Transporter Assays)

Perform assay according to established protocols.

References

1. Gripon, P. *et al.*, Infection of a human hepatoma cell line by hepatitis B virus. *Proc. Natl. Acad. Sci. USA*, **99**, 15655-15660 (2002).
2. Parent, R. *et al.*, Origin and characterization of a human bipotent liver progenitor cell line. *Gastroenterology*, **126**, 1147-1156 (2004).
3. Andersson, T.B. *et al.*, 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 (2012).
4. Kanebratt, K.P., and Andersson, T.B., HepaRG cells as an *in vitro* model for evaluation of cytochrome P450 induction in humans. *Drug Metab. Dispos.*, **36**, 137-145 (2008).
5. McGill, M.R. *et al.*, HepaRG cells: a human model to study mechanisms of acetaminophen hepatotoxicity. *Hepatology,* **53**, 974-982 (2011).
6. Le Hegarat, L. *et al.*, 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 (2010).

HepaRG is a trademark of BioPredic International.
CompoZr is a registered trademark of Sigma-Aldrich Co. LLC.
Corning and Matrigel are registered trademarks of Corning, Inc.
GlutaMAX is a trademark of Life Technologies Corp.

MDM,AA,MAM,DA 09/15-1

These products are covered by the “Consumable License” as described in Exhibit 1 and the HepaRG™ LULL described in Exhibit 2.

EXHIBIT 1

LICENSE AGREEMENT - ADME/TOX CELL LINES

This Product and its use are the subject of one or more of the following patents controlled by Sangamo BioSciences, Inc.: U.S. Patent Nos. 6,534,261, 6,607,882, 6,746,838, 6,794,136, 6,824,978, 6,866,997, 6,933,113, 6,979,539, 7,013,219, 7,030,215, 7,220,719, 7,241,573, 7,241,574, 7,585,849, 7,595,376, 6,903,185, 6,479,626, US20030232410, US20090203140 and corresponding foreign patent applications and patents.

BEFORE OPENING OR USING THIS PRODUCT, PLEASE READ THE TERMS AND CONDITIONS SET FORTH IN THIS LICENSE AGREEMENT. YOUR USE OF THIS PRODUCT SHALL CONSTITUTE ACKNOWLEDGMENT AND ACCEPTANCE OF THESE TERMS AND CONDITIONS. If you do not agree to use this Product pursuant to the terms and conditions set out in this License Agreement, please contact Sigma Technical Services within ten days of receipt to return the unused and unopened Product for a full refund; provided, however, that custom-made Products may not be returned for a refund.

The purchase of this Product conveys to you, the buyer, the non-transferable right to use the purchased Product for Licensed Research Use (see definition below) subject to the conditions set out in this License Agreement. If you wish to use this Product for any purpose other than Licensed Research Use, you must first obtain an appropriate license (see information set out below).

This Product may not be used for any purpose other than Licensed Research Use. Product, as used herein, means the Cell Line and any derivative cells or cell lines created by the buyer which contain and/or incorporate genetic information derived from the Cell Line. Your right to use this Product for Licensed Research Use is subject to the following conditions and restrictions:

The type of license you have subject to this Agreement (Evaluation, Annual, Extended and Consumable) is listed on the outside of the package and/or on the invoice you received from us.

1. “Licensed Research Use” means any use for research purposes, other than:

(a) Licensing, selling, distributing, or otherwise providing the product or modified versions of it to any third party other than Sigma and its affiliates as provided herein: provided however, that you may provide the product or modified versions of it to researchers within your research organization located at the same research facility or campus who are similarly bound to the use restrictions herein;

(b) GMP production of therapeutic, diagnostic, prophylactic or other medicinal products intended for use in humans or non-human animals, or any other industrial use solely to the extent involving commercial sale of a product or service. If a molecule or any derivative of such molecule is used in or administered to humans, then the production of such molecule shall be deemed to be GMP production and therefore in violation of this License Agreement;

2. You may not transfer the Product, its components, or any materials made through the use of this Product, including further modified cells, to any third party without prior written approval of Sigma. Notwithstanding the foregoing, the Product or materials made through use of the Product may be transferred by you without such prior written approval to your legal affiliates or bona fide third party contractors performing paid work on your behalf, provided the use by such third party contractors is limited to performance of work for you and any results or product of such work shall not be shared by the third party contractor with any other person.

3. For Products covered by an “Evaluation” License, this Agreement shall expire ninety (90) days following your receipt of the Product.

4. For Products covered by an “Annual” License, this Agreement shall expire three hundred and sixty-five (365) days following your receipt of the Product.

5. For Products covered by an “Extended” License, this Agreement shall remain in force in perpetuity following your receipt of the Product.

6. For Products covered by a “Consumable” License, you may:

(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.

(b) Use Products for the provision of commercial services to a third party for monetary gain, but limited to only delivery of research results and data from use of the Product, and not the Product, its components, or any materials made through the use of this Product, including further modified cells.

7. 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.

8. Your right to use the Product will terminate immediately upon expiration of this License Agreement, or in the event that you fail to comply with these terms and conditions. You shall, upon such termination of your rights, destroy all Product, any modified versions of the Product, and components thereof in your control, and notify Sigma of such in writing.

For information on purchasing a license to this Product for purposes other than Licensed Research Use, contact your local Sigma Sales representative, who will refer you to the proper licensing representative, or in the USA call 800-325-3010.

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.

©2015 Sigma-Aldrich Co. LLC. All rights reserved. SIGMA-ALDRICH is a trademark of Sigma-Aldrich Co. LLC, registered in the US and other countries. Sigma brand products are sold through Sigma-Aldrich, Inc. Purchaser must determine the suitability of the product(s) for their particular use. Additional terms and conditions may apply. Please see product information on the Sigma-Aldrich website at www.sigmaaldrich.com and/or on the reverse side of the invoice or packing slip.