

## Product Information

### HepaRG™ Assay Ready Plates containing HepaRG™ B-CLEAR® OATP1B3 KO Cells

Catalog Number MTOX1016QP24

## 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](http://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.<sup>1</sup> 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.<sup>2</sup>

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.<sup>3-6</sup> 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 B-CLEAR® OATP1B3 KO Cells. They are intended for use with HepaRG B-CLEAR 5F Clone Control Cells (Catalog Number MTOX1010Q) 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 24 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 24-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 MTOXHFSISUP), 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 Friday)

1. Remove the old medium from each well.
2. Add 500 µl of fresh Recovery Medium into each well. Put the plates back into incubator to allow ≥3 days of recovery (over the weekend).

#### Day 4 (Monday)

1. Aspirate the old medium and replace with Maintenance Medium.
2. Repeat on Day 6 (Wednesday).

#### Day 8 (Friday)

1. Remove Maintenance Medium from each well.
2. Add 500 µl of fresh Pre-induction Medium into each well. Put the plates back into incubator to incubate over the weekend.

#### Day 11 (Monday)

1. Remove Pre-induction Medium from each well.
2. Add 500–1,000 µl of test article in Serum Free Induction Medium to each well.
3. Refresh with test article in Serum Free Induction Medium on Days 12 and 13 (Tuesday and Wednesday, respectively).

#### Day 14 (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 1 ml 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 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 (Perform Transporter Assays)

Perform assay according to established protocols.

## C. Protocol for calculation of Biliary Excretion Index

The methodology and formula for calculation of biliary excretion index (BEI), using B-CLEAR technology, were adopted from Swift et al., 2010.<sup>7</sup>

## 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).
7. Swift, B. *et al.*, (2010) Sandwich-Cultured Hepatocytes: An *In Vitro* Model to Evaluate Hepatobiliary Transporter-Based Drug Interactions and Hepatotoxicity. *Drug Metab. Rev.*, August, **42**(3), 446–471.

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

MDM,AA,MAM,DA 11/15-1

These products are covered by the “Consumable License” as described in Exhibit 1, the HepaRG™ LULL described in Exhibit 2 and limited-use license for B-CLEAR® system in Exhibit 3.

**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.

## EXHIBIT 3

### LIMITED-USE LICENSE FOR B-CLEAR® SYSTEM FOR THE UNITED STATES OF AMERICA AND NORTH AMERICA

This is a binding legal agreement (this “**License**”) between you (“**Customer**” or “**You**”), and Qualyst Transporter Solutions, LLC (“**Qualyst**”) regarding the enclosed B-CLEAR® System (this “**Product**”). Carefully read this License prior to opening the sealed package. **BY OPENING THE SEALED PACKAGE, YOU AGREE TO BE BOUND BY THE TERMS OF THIS LICENSE.** If You do not agree to the terms of this License, and You have not opened the sealed package, promptly notify Qualyst in writing and return the unopened Product and all accompanying items (including all written material and containers) to Qualyst within five (5) business days.

This License sets forth Customer’s rights to use this Product. The meaning of “Product” hereafter shall include any component parts thereof and any documentation made available for use of the B-CLEAR® system. Upon receipt of the applicable fee, Qualyst grants to Customer the personal, non-exclusive, and non-transferable right to use this Product in the United States of America and North America. Customer is solely responsible for the use of this Product by any person.

Customer acknowledges and agrees that the manufacture, use, sale or import of this Product may be subject to one or more issued or pending U.S., E.U. and other worldwide patents or patent applications and/or corresponding foreign equivalents owned or controlled by Qualyst. Customer acknowledges and agrees that the documentation and components included in the Product are the proprietary and copyrighted material of Qualyst. The purchase and use of this Product shall be governed under Qualyst’s standard Terms and Conditions, and any other written agreement between the parties, and such terms and agreements are incorporated herein by reference. Your purchase of this Product, subject to this License, conveys to Customer the non-transferable right to use the purchased amount of the Product in this shipment and components of the Product in this shipment in research conducted by Customer, such right to expire upon the completion of experiments from this shipment.

Customer shall not sell or otherwise transfer (a) this Product (b) any components of this Product; or (c) any materials made using this Product or its components, and Customer shall not otherwise use this Product or its components for Commercial Purposes. “**Commercial Purposes**” means: (1) use of this Product in manufacturing; (2) use of this Product, in exchange for fees or other consideration paid or payable by an unrelated party, to provide services, information, or data; (3) use of this Product for therapeutic, diagnostic or prophylactic purposes; or (4) resale of this Product, whether or not such Product is resold for use in research. For clarification, Customer may use and transfer information or materials made through the use of this Product to support Customer’s research in any manner Customer deems necessary or appropriate, provided that in no event may Customer use the Product to provide services.

Subject to the terms of this License, Qualyst will not assert a claim against Customer alleging infringement based upon the manufacture, use, or sale of a therapeutic, clinical diagnostic, vaccine or prophylactic product developed by Customer in which this Product was employed, provided that neither this Product nor any of its components was used in the manufacture of such Product.

For information on obtaining a license to use this Product for purposes beyond the scope of this License, contact **Qualyst Transporter Solutions, LLC, Attn: Corporate Development, 2810 Meridian Parkway, Suite 100, Durham, NC 27713; +1-919-313-6500 (phone); +1-919-313-0163 (fax), or Sigma-Aldrich, Attn: Customer Support, PO Box 14508, St Louis, Mo 63178; +1-800-325-3010 (Phone); +1-800-325-5052 (Fax)**