Product Information

HepaRG™ Assay Ready Plates containing HepaRG MRP3 Knockout Cells

Catalog Number MTOX1105P48

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. 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.

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.

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 MRP3 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 48 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 48-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 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 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 500 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 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 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 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.

References

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