

# Corning<sup>®</sup> Cryopreserved HepatoCells

## Instructions for Use

Corning<sup>®</sup> cryopreserved HepatoCells are derived from single-donor human hepatocytes using proprietary technology for immortalizing primary cells. HepatoCells retain the physiological properties of their parental hepatocytes, show mature hepatocyte-like morphology, and express the metabolic enzymes, nuclear receptors, and drug transporters common to mature hepatocytes. These cells have been characterized for CYP3A4, CYP 1A2, and CYP 2B6 induction response to prototypical inducers, as well as cytotoxicity applications.

Cryopreserved HepatoCells were developed to overcome some of the shortcomings of primary human hepatocytes such as large lot-to-lot variability, short life span, tendency to dedifferentiate in culture, and limited supply of high quality materials. With virtually unlimited supply and consistent performance, cryopreserved HepatoCells can work as a reliable alternative *in vitro* hepatic model for ADME/Tox screening by providing the convenience of being readily available and supporting long-term studies with large quantities of cells.

### Process Overview

This instruction for use describes the procedure for thawing and plating cryopreserved HepatoCells to form a monolayer culture and the procedure for performing CYP450 induction assay with plated cultures. One vial contains  $\geq 8$  million viable cells, enough for one 96-well plate or one 24-well plate when following the procedure.

**Note:** Corning cryopreserved HepatoCells have been validated for CYP induction application; although we are in the process of developing other applications, users are welcome to test the samples for any applications of their interest. Corning cryopreserved HepatoCells show typical mature hepatocyte morphology after 3 to 4 days in culture.

### Caution

When working with cryopreserved HepatoCells, follow the same safety recommendations as working with primary human hepatocytes (i.e., treat the cells as potential biohazards). Always wear suitable personal protective equipment such as latex gloves, safety glasses and/or a face shield, and a polyester lab coat while working with these cells. The steps involved with the cell culture should be carried out in a sterile biological safety cabinet and standard practices for biohazard materials apply.

### Materials Supplied

#### Dry shipper

Corning cryopreserved HepatoCells (Corning Cat. No. 354881):  $\geq 80\%$  viability,  $\geq 8$  million cells/vial. Store cells in vapor phase of liquid nitrogen tank immediately upon receipt.

### Materials and Equipment to be Ordered or Supplied by the User

- Corning culture medium for HepatoCells (CMH) (Corning Cat. No. 354882)
- Corning Matrigel<sup>®</sup> basement membrane matrix, phenol red-free (Corning Cat. No. 356237)
- Antibiotics, such as Penicillin-Streptomycin solution (100x) (Corning Cat. No. 30-002-CI)
- Fetal Bovine Serum, heat inactivated (Corning Cat. No. 35-016-CV)
- Corning<sup>®</sup> BioCoat<sup>™</sup> Collagen I-coated 96-well plates (Corning Cat. No. 354407)
- Certified tissue culture hood
- Phase-contrast microscope
- Cell culture incubator (5% CO<sub>2</sub>, 37°C, 95% humidity)
- Water bath (37°C)
- Liquid nitrogen storage unit

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- Refrigerator or equivalent storage chamber (4°C)
- Centrifuge capable of achieving 150 x g
- Aspirating device (i.e., pipettor, vacuum aspirator)
- Falcon 50 ml Polypropylene Conical Tubes (Cat. No. 352070)
- Multi-channel pipets and reagent reservoirs
- Materials or equipment for cell count

**Preparation Before Use**

1. All the media used in the protocol is recommended to be supplemented with Penicillin-Streptomycin solution (1:100 dilution).
2. Prepare plating medium by adding FBS (final concentration of 10%) to CMH. Note that serum is only needed for the plating step, therefore, DO NOT add FBS to the entire bottle of CMH.
3. Pre-warm plating medium in a 37°C water bath.

**Protocol for Thawing Corning Cryopreserved HepatoCells**

1. For each vial of cells, aliquot 10 to 15 mL of warm plating media into a 50 mL conical centrifuge tube.
2. Remove a cryovial of Corning cryopreserved HepatoCells from liquid nitrogen storage unit and put in dry ice.
3. Transfer the cryovial into 37°C water bath immediately.
4. Thaw the cryovial for 1 minute (no more than 2 minutes) by gently moving the vial back and forth in the water bath to facilitate heat transfer, until there is a small ball of frozen cells remaining in the vial.
5. Remove the cryovial from the water bath and quickly transfer the cryovial to a tissue culture hood.
6. Wipe the cryovial with a 70% alcohol pad before removing lid.
7. Pour the vial content to the 10 to 15 mL pre-aliquoted plating medium in the 50 mL tube.
8. Rinse the cryovial with 1 mL warm plating medium and transfer the rinse medium back to the above 50 mL tube.
9. Use a 10 mL serological pipet to break any large cell clumps by quickly pipetting up and down. It is fine to have some small cell clumps at this step.
10. Add plating medium to the centrifuge tube until the solution volume is equal to 50 mL.
11. Centrifuge at 150 x g for 10 minutes at room temperature.
12. Aspirate supernatant carefully without disturbing the pellet and leave a small amount of medium (~0.5 mL) in the tube.
13. Add about 5 mL of plating medium to resuspend the pellet.
14. Use a 10 mL serological pipet to break any cell clumps by quickly pipetting up and down 10 to 20 times to make a homogeneous cell suspension. It is important to have homogeneous cell suspension at this step for accurate cell counting.
15. Count cell number to determine viability and cell concentration.
16. Adjust cell suspension volume with plating medium to achieve desired cell concentration (see Table 1 for seeding density).

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**Table 1. Seeding Density and Medium Volume for Different Plate Formats**

Plate Format	Seeding Density, Cell no. per Well	Medium Volume per Well
24-well	0.4x10 <sup>6</sup>	0.5 mL
96-well	0.8x10 <sup>6</sup>	0.1 mL

**Note:** Medium volume in Table 1 applies to cell seeding, Corning Matrigel® matrix overlay, medium change, induction treatment, and substrate incubation during enzymatic assay.

## Protocol for Performing CYP450 Induction Assay Using Corning Cryopreserved HepatoCells

### Day 1 Morning – Cell Seeding

1. In the morning, thaw cells as instructed above in the protocol for thawing Corning cryopreserved HepatoCells.
2. Further dilute the cells with warm plating medium to a desired cell concentration (e.g., 0.8 x 10<sup>6</sup> cells/mL).
3. Seed cells into Corning BioCoat™ Collagen I-coated 96-well plate(s) using a multi-channel pipet according to Table 1.
4. Incubate the plate overnight in a cell culture incubator.

### Day 1 Afternoon – Matrigel Overlay

**Note:** Corning Matrigel matrix stock vial should be thawed overnight at a 4°C refrigerator the day before use.

1. Pre-chill pipet/container that will be used to make Matrigel solution.
2. Make 0.25 mg/mL Matrigel solution with cold CMH using a chilled pipet. Keep the Matrigel solution cold.

**Note:** Make sure all the pipets/containers in contact with the Matrigel stock are kept cold to avoid gelling of the Matrigel matrix solution.

3. Four to six hours after plating HepatoCells, take out the plates from the incubator, remove plating medium, and add cold Matrigel solution at the volume indicated in Table 1.
4. Transfer the plate back to the cell culture incubator.

### Day 2 to Day 4 – Induction Treatment

1. Pre-warm CMH in 37°C water bath.
2. Change medium daily with warm CMH containing either test articles, positive control inducers, or solvent vehicle control at the volume indicated in Table 1. See Table 2 for suggested concentrations of positive control inducers and solvent vehicle control.

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**Table 2. Concentrations of Positive Control Inducers**

Enzyme	Positive Control Inducer	Stock Ssolution	Final Concentration
CYP3A4	Rifampicin	10 mM in DMSO	10 $\mu$ M in CHM
CYP1A2	Omeprazole	50 mM in DMSO	50 $\mu$ M in CHM
CYP2B6	Phenobarbitol	0.1 M in 10% DMSO	1 mM in CHM
Solvent control vehicle		100% DMSO	0.1% in CHM

**Day 5 - Enzymatic Assay or Gene Expression Analysis****For enzymatic assays:**

1. Incubate cells with probe substrate(s) in pre-warmed CMH.
2. Refer to Table 3 for recommended substrate concentration and incubation time.

**Table 3. Substrate Concentration and Incubation Time for Enzymatic Assay**

Enzyme	Substrate	Stock Solution	Final Concentration	Substrate Incubation Time
CYP3A4	Testosterone	0.1 M in DMSO	200 $\mu$ M in CMH	60 min
CYP1A2	Phenacetin	0.1 M in DMSO	100 $\mu$ M in CMH	60 min
CYP2B6	Bupropion	0.05 M in Methanol	250 $\mu$ M in CMH	60 min

3. At the end of enzymatic assay, collect sample, and mix with stop solution.
4. Analyze metabolite with HPLC or LC-MS/MS method.

**For gene expression analysis:**

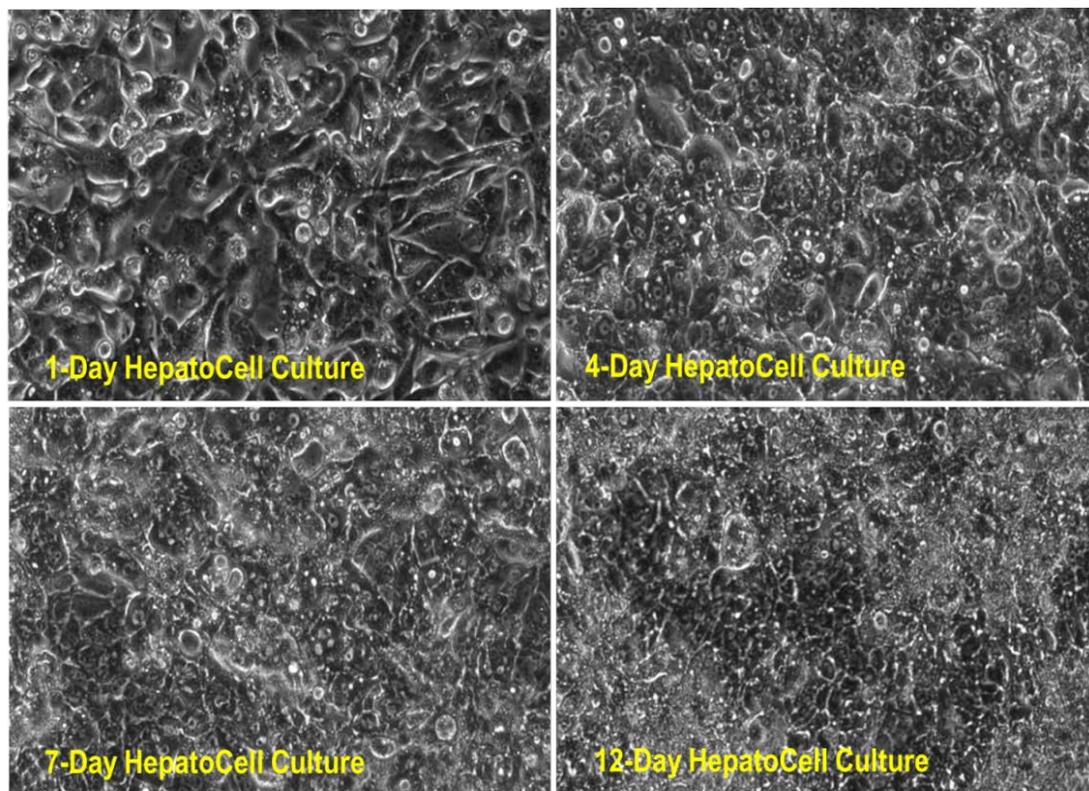
1. Isolate RNA using your preferred isolation method.
2. Perform RT-PCR to determine basal and induced expression level of CYP450 genes of your interest.

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## Corning HepatoCells Morphology in Plated Culture

Corning HepatoCells show mature hepatocyte morphology after 3 to 4 days of culture in Corning BioCoat™ Collagen I-plates, such as cuboidal cell shape, bi-nucleation, or multi-nucleation typical to mature primary hepatocytes, distinct nucleoli, and bile canaliculi. Corning HepatoCells can be cultured for more than 2 weeks (Figure 1)..



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12. Shrink Wrap License Agreement for Corning HepatoCells

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Revision History				
Rev.	Change No.	Description of Change	Revised By	Revised Date
1	CC 03126	Initial issue	R. Zuo M. Andes	05/15/2015

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