Corning® Cryopreserved HepatoCells
Instructions for Use

Corning® 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 >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): >80% viability, >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® 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® BioCoat™ Collagen I-coated 96-well plates (Corning Cat. No. 354407)
- Certified tissue culture hood
- Phase-contrast microscope
- Cell culture incubator (5% CO₂, 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).
Table 1. Seeding Density and Medium Volume for Different Plate Formats

<table>
<thead>
<tr>
<th>Plate Format</th>
<th>Seeding Density, Cell no. per Well</th>
<th>Medium Volume per Well</th>
</tr>
</thead>
<tbody>
<tr>
<td>24-well</td>
<td>$0.4 \times 10^6$</td>
<td>0.5 mL</td>
</tr>
<tr>
<td>96-well</td>
<td>$0.8 \times 10^5$</td>
<td>0.1 mL</td>
</tr>
</tbody>
</table>

**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., $0.8 \times 10^6$ 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.
Table 2. Concentrations of Positive Control Inducers

<table>
<thead>
<tr>
<th>Enzyme</th>
<th>Positive Control Inducer</th>
<th>Stock Solution</th>
<th>Final Concentration</th>
</tr>
</thead>
<tbody>
<tr>
<td>CYP3A4</td>
<td>Rifampicin</td>
<td>10 mM in DMSO</td>
<td>10 µM in CHM</td>
</tr>
<tr>
<td>CYP1A2</td>
<td>Omeprazole</td>
<td>50 mM in DMSO</td>
<td>50 µM in CHM</td>
</tr>
<tr>
<td>CYP2B6</td>
<td>Phenobarbitol</td>
<td>0.1 M in 10% DMSO</td>
<td>1 mM in CHM</td>
</tr>
<tr>
<td>Solvent control vehicle</td>
<td></td>
<td>100% DMSO</td>
<td>0.1% in CHM</td>
</tr>
</tbody>
</table>

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

<table>
<thead>
<tr>
<th>Enzyme</th>
<th>Substrate</th>
<th>Stock Solution</th>
<th>Final Concentration</th>
<th>Substrate Incubation Time</th>
</tr>
</thead>
<tbody>
<tr>
<td>CYP3A4</td>
<td>Testosterone</td>
<td>0.1 M in DMSO</td>
<td>200 µM in CMH</td>
<td>60 min</td>
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<tr>
<td>CYP1A2</td>
<td>Phenacetin</td>
<td>0.1 M in DMSO</td>
<td>100 µM in CMH</td>
<td>60 min</td>
</tr>
<tr>
<td>CYP2B6</td>
<td>Bupropion</td>
<td>0.05 M in Methanol</td>
<td>250 µM in CMH</td>
<td>60 min</td>
</tr>
</tbody>
</table>

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.
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-nucelation, 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

Please read carefully the following legally binding End-User License Agreement between Corning Incorporated and you for the use of Corning HepatoCells. These cells are for research purposes only. By breaking the seal on this package, you, the End-User, agree not to redistribute, resell, transfer, subculture, reverse engineer, modify, improve, derivatize, or subclone this cell line without first obtaining express written permission from Corning Incorporated. Furthermore, this cell line cannot be used for the propagation of viruses, the identification of new drug targets, or the production of proteins or vaccines without first obtaining a license from Corning Incorporated. Nothing in this End-User Agreement provides you with any rights whatsoever under patents or trade secrets of Corning Incorporated.
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<th>Description of Change</th>
<th>Revised By</th>
<th>Revised Date</th>
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<td>Initial issue</td>
<td>R. Zuo M. Andes</td>
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