Corning® Hepatocyte Maintenance Medium for Cell Plating, Induction, and Hepatotoxicity Studies

Assay Protocol

CORNING

Materials

- Corning hepatocyte maintenance medium (Corning Cat. No. 40-550-CV)
- Cryogenic preserved primary human hepatocytes
- Cryogenic Preserved Hepatocyte Recovery Medium (CHRM)
- Collagen I-coated plates (Plate format options: 6-well plate, Corning Cat. No. 356400; 12-well plate, Corning Cat. No. 354500; 24-well plate, Corning Cat. No. 354408, and 96-well microplate, Corning Cat. No. 354407)
- Corning® Matrigel® matrix (Corning Cat. No. 356237)
- Multi-channel pipettors and tips
- Vi-CELL® cell viability analyzer (Beckman Coulter)
- Cell culture incubator (5% CO_2, 37°C, 95% humidity)
- Certified biosafety cabinet

Procedure for plating primary human hepatocytes

Day 1 (cell plating)

Step 1: Prepare hepatocyte plating medium by supplementing Corning hepatocyte maintenance medium with 10% FBS.

Step 2: Thaw a vial of cryogenic preserved primary human hepatocytes using CHRM medium according to the manufacturer's protocol.

Step 3: Re-suspend the resulting hepatocyte pellet in plating medium prepared in step 1.

Step 4: Estimate the density and percentage of viable cells by the trypan blue exclusion method using the Vi-CELL analyzer.

Step 5: Dilute the cells from previous steps with plating medium to a desired seeding density.

Step 6: Pipet cells onto a Corning Collagen I-coated plate(s) according to Table 1.

Plate	Viable Cells/Well	Volume/Well
6-well	1.4×10^6	2.0 mL
12-well	0.7×10^6	1.0 mL
24-well	0.35×10^6	0.5 mL
96-well	0.06×10^6	0.1 mL

Table 1. Recommended densities for plating hepatocytes

Step 7: Transfer the plates to the cell culture incubator and allow cells to attach to the plates for 6 hours.

Step 8: At the end of 6 hours, replace the plating medium with pre-warmed Corning hepatocyte maintenance medium.

Step 9: Optional Matrigel matrix overlay method: at the end of 6 hours, add the same volume of cold medium (approximately 4°C) containing 0.25 mg/mL Matrigel matrix to each well.

Step 10: Return the plates to the incubator for further experiments as required.

Procedure for CYP induction studies

Day 2 to 4 (3-day induction)

Step 11: Remove the culture medium from each well and replace with pre-warmed Corning® hepatocyte maintenance medium containing a vehicle control, a positive inducer (e.g., rifampicin for CYP3A4, Omeprazole for CYP1A2, Phenobarbital for CYP2B6, etc.), or test compounds.

Step 12: Return the plates to the incubator overnight.

Step 13: Change the medium with test compounds or a positive inducer once daily for three consecutive days.

Day 5

Step 1: Evaluate CYP enzymatic activity using preferred methods.

Step 2: Isolate total RNA if gene expression analysis is needed.

Day 1	Thaw and plate cells Corning Matrigel® matrix overlay (optional)
Day 2	Change to induction medium
Day 3	Change to induction medium
Day 4	Change to induction medium
Day 5	Evaluate CYP enzymatic activity Isolate RNA for gene expression analysis

Table 2. Recommended schedules for induction study

Procedure for hepatotoxicity studies

Day 2

Step 1: Remove the culture medium from each well and replace with pre-warmed Corning hepatocyte maintenance medium that contains test compounds and vehicle controls.

Step 2: Return the plates to the incubator overnight.

Day 3

Perform cytotoxicity assay using preferred methods.

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