

## Assay Methods Hepatocyte Protocols

### Plating of BD Gentest™ Inducible-Qualified Human CryoHepatocytes

The protocol below describes the procedure for plating cryopreserved hepatocytes on

BD BioCoat™- Collagen I Coated plates once they have been thawed and recovered.

Note: Follow standard sterility practices for cell culture.

#### Materials and Equipment:

- ISOM's media (BD Biosciences Cat No. 454600), or customer preferred seeding media
- Fetal Bovine Serum (FBS)
- ISOM's Seeding Media: ISOM's media + 10% FBS
- BD Hepatocyte Culture Media Kit (BD Cat. No. 355056) [Note: to prepare Hepatocyte Culture Media, combine 500 mL of Hepatocyte Defined Media (supplied with kit), 5 micrograms epidermal growth factor (supplied with kit), 5 mL 200 mM (29.2 mg/ml) sterile l-glutamine (purchase separately), 0.5 mL 50 mg/mL sterile gentamicin sulfate (purchase separately) and 1.5 mL 0.25 mg/mL Fungizone (purchase separately).], or customer preferred hepatocyte culture media
- Serological pipet 5 mL or 10 mL (BD Bioscience Cat, No. 357543 or 357551)
- Repeat pipet and tips or Multi-channel pipette and tips
- 24-well collagen I coated plate, BD Cat. Nos. 356408 (50 plates) / 354408 (5 plates), 48-well collagen I coated plate, BD Cat. Nos. 356505 (50 plates) / 354505 (5 plates), 96-well collagen I coated plate, BD Cat. Nos. 356405 (50 plates) / 354405 (5 plates)
- Biosafety hood
- Cell Culture Incubator (with CO<sub>2</sub> atmosphere appropriate for the culture media (5% for ISOM's media)

#### Procedure

1. Pre-warm ISOM's Seeding Media containing 10% FBS to 37°C
2. Thaw and determine the viable cells per mL and total viable cell recovery using a BD recommended method or other methods as preferred.
3. Dilute cells with pre-warmed ISOM's Seeding Media to 1 x 10<sup>6</sup> cells/mL in 50 mL conial tube (BD Biosciences Cat. No. 352077). (Note: seeding density may be optimized in the user's laboratory for specific lots of hepatocytes.)

**Chart 1.** Recommendation of plating Human CryoHepatocytes used for cytochrome P450 induction assays

| Plate   | Vol. of each well | Cells/well |
|---------|-------------------|------------|
| T25     | 5000µL            | 5,000,000  |
| 6-well  | 2000µL            | 1,920,000  |
| 12-well | 800µL             | 800,000    |
| 24-well | 400µL             | 400,000    |
| 48-well | 150µL             | 150,000    |
| 96-well | 60µL              | 60,000     |

4. Cap the above mentioned 50 mL tube tightly and gently invert tube 2 to 3 times to suspend the cells evenly. Be careful not to introduce air bubbles into the hepatocyte suspension. Use repeat pipet or multichannel pipet to dispense cells according to the volumes in Chart 1 above. (Note: hepatocytes are sensitive to shear, do not use excessive pipetting speeds.)
5. After cells plating is completed, gently move the plate in a star pattern on a level surface to distribute the cells evenly over the bottom of the cell. See figure below. (Note: swirling will cause the cells to accumulate excessively in the center of the well and can cause cell death due to anoxia.)
8. After 2 to 4 hours, gently aspirate the ISOM's Seeding Media and gently refeed cells with complete Hepatocyte Culture Media (or customer preferred hepatocyte culture media). Refer to chart 1 for the amount of media to be added into each well. Take care not to dislodge the cells when adding the media.
9. Keep plates in the incubator overnight for further experiments as required.
10. After overnight incubation, tap plate gently to dislodge any dead cells on the top of the monolayer and re-feed cells with complete Hepatocyte Culture Media (or customer preferred hepatocyte culture media). Note: you may wish to take digital picture to document cell morphology and the degree of monolayer confluence.



6. Place the plates level in a 37°C, 5% CO<sub>2</sub> incubator.
7. It is suggested that every 20 to 30 minutes during the first 2 hours plating, remove plates from incubator and gently rock the plates as described in Step 5 to redistribute the cells evenly in the wells. Gently tapping the edge of the plate may also be helpful to redistribute the cells. As noted in step 5, excessive accumulation of cells in the center of the wells can cause cell death.

Note: If performing a cytochrome P450 induction assay with the plated hepatocytes, one day 2 after plating, replace the Hepatocyte Culture Media with Hepatocyte Culture Media containing the desired concentration(s) of inducer (or solvent vehicle). Replace the Hepatocyte Culture Media with inducer (or solvent vehicle) daily for the duration of the inducer treatment.