# Corning® Gentest™ High Viability CryoHepatocyte Recovery Kit

## Protocol - Hepatocyte Assay Methods

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### Purpose

The protocol below describes the procedure for thawing and recovering cryopreserved human hepatocytes using the Corning Gentest High Viability CryoHepatocyte Recovery Kit, which includes Corning Gentest High Viability Recovery Medium and Corning Gentest Plating Medium (also available separately).

**Note:** Please follow standard sterility practices for cell culture.

### Materials

- Corning Gentest High Viability CryoHepatocyte Recovery Kit (Corning Cat. No. 454534)
- Corning Gentest High Viability CryoHepatocyte Recovery Medium (Corning Cat. No. 454560)
- Corning Gentest CryoHepatocyte Plating Medium (Corning Cat. No. 454561)
- Fetal Bovine Serum (FBS)
- 37°C water bath
- Low speed centrifuge at room temperature (swinging bucket model that can accommodate 50 mL tubes, e.g., Eppendorf Centrifuge Model 5810)
- Biosafety hood
- Trypan Blue (Sigma Cat. No. T8154)
- Hemacytometer
- Microscope
- 1X Phosphate Buffered Saline (PBS)
- 2 mL and 5 mL Falcon® serological pipets (Corning Cat. Nos. 357507 and 357543)
- Corning BioCoat™ Collagen I Multiwell Plates
- Corning Hepatocyte Culture Media, (Corning Cat. No. 355056), and recommended supplements (or customer preferred culture media for conducting induction or metabolism assays)

### Procedure

1. Remove one tube each of recovery media and plating media from the kit container and transfer to a biosafety hood.
2. Add 5 mL of FBS to 45 mL of plating media.
3. Pre-warm both the recovery media and plating media (with FBS) to 37°C using a 37°C water bath.
4. Store cryovials in liquid nitrogen vapor until ready to use.
5. Remove the cryovials from liquid nitrogen and immediately transfer to a 37°C water bath. Transferring the cryovials from shipper to water bath should take no more than 10 seconds.

**Note:** Do not aliquot the 45 mL of recovery media to smaller volumes when processing multiple vials. Up to 5 vials of cryopreserved hepatocytes can be processed in one purification. Add the contents of each cryovial directly to the total 45 mL of recovery media. Using smaller volumes of the recovery media will result in reduced viability.

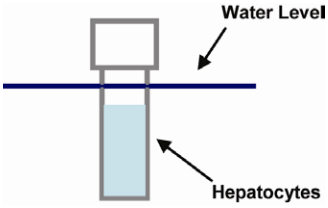


**Figure 1.** Cryovial cap should remain above water level.

6. Place the cryovials into the water bath, but do not completely submerge. Be careful to keep the cap above the water (Figure 1). Gently shake the cryovial back and forth to achieve even thawing while continuously monitoring the contents. When only a few small ice crystals are remaining, remove the cryovial from the water bath. Thaw time should be no more than 2 minutes.
7. Remove the cryovials from the water bath and wipe with a 70% Ethanol-saturated towel. Return the cryovials to the biosafety hood.

**Note:** Proceed immediately to the next step, as delays can decrease viability.

8. Carefully decant the 1.5 mL of semi-thawed hepatocytes into the recovery media tube.
9. Rinse the empty cryovial tube with approximately 1 mL solution from the recovery media tube and pour back into the recovery media tube.
10. Mix the hepatocytes with recovery media by gently inverting the 50 mL recovery media tube two to three times. Proceed immediately to the centrifugation steps without delay.
11. Centrifuge 50 mL recovery media tube with hepatocytes at 100 x g for 10 minutes at room temperature.
12. Centrifuge Acceleration and Brake Settings: The acceleration rate of the centrifuge should be set to the medium setting, and the brake should be set to the lowest or "off" setting. For example, if using an Eppendorf table top centrifuge model 5810, the acceleration should be set to "5" and the brake should be set to "0" (maximum settings are "9").
13. Very gently remove the recovery media tube immediately after centrifugation and transfer it to the biosafety hood. A cloudy layer may be noted at the top of the recovery media tube. The hepatocyte pellet should be visible at the bottom of the tube. Care should be taken not to disturb either the top layer or the hepatocyte pellet while handling the tube. Carefully aspirate and discard the supernatant fluid without disturbing the hepatocyte pellet.
14. Resuspend the hepatocyte pellet in approximately 1 to 2 mL of plating media (containing FBS). Gently resuspend with a 2 mL pipet and titrate two to three times very gently to obtain a homogeneous cell suspension. Measure and record the actual resuspension volume with a 2 mL pipet.

**Note:** Formation of air bubbles indicates the titration force is excessive and can adversely impact cell viability.

15. Keep thawed cells at room temperature and determine cell viability and recovery immediately by following the protocol entitled "Corning® Gentest™ Determination of Cell Viability Protocol."

**Corning acquired the BioCoat™, Falcon®, and Gentest™ brands.**
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