

Corning® HepGo™ Assay-ready 3D Liver Spheroid Kit

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Instruction for Use

Customers are encouraged to read through the user guide in advance, and contact your local Corning Sales Specialist for assistance if necessary.

Kit Components/Storage Conditions

Description		Quantity	Storage Temp.
Corning HepGo Assay-ready 3D Liver Spheroid Kit (Cat. No. 454660)	96-well Corning Assay-ready Liver Spheroids made from single donor primary human hepatocytes	2	N/A
	Corning HepGo Hepatocyte Culture Medium (also referred to as Kit Companion Medium; Cat. No. 454670), serum-free, antibiotic-free hepatocyte culture medium	1 x 100 mL	2°C to 8°C
	Temperature Logger and return envelope	1 each	N/A

Materials and Equipment Required (not included in the kit)

- ▶ 70% ethanol or 70% isopropanol
- ▶ Centrifuge with adaptors for microplates (96-well)
- ▶ 37°C tissue culture incubator
- ▶ 37°C water bath
- ▶ Microscope or automated imager
- ▶ Sterile 50 mL reagent reservoir
- ▶ Multi-channel pipettor and 200 µL tips
- ▶ Sterile 10, 20, 200, 1000 µL pipets and pipet tips
- ▶ Stainless steel tweezers or forceps with serrated tips (autoclaved or thoroughly sprayed with 70% ethanol)

Corning HepGo Hepatocyte Culture Medium Order and Preparation

Corning HepGo Hepatocyte Culture Medium (100 mL) is provided in each kit package. It is also provided as a separate product for customer order. Based on specific assay protocol, customers need to calculate the amount of medium needed for using the assay-ready liver spheroid kit. In general, half medium change of 100 µL/well (i.e., 12 mL/microplate) is needed every other day for liver spheroid culture maintenance.

Pending the medium need for specific applications, we recommend customers to order additional Corning HepGo Hepatocyte Culture Medium (Cat. No. 454670, 100 mL aliquots) in advance if necessary. This medium can be shipped separately on dry ice and should be stored at -20°C immediately upon receiving for long-term storage.

One day before the planned use of the medium, remove frozen medium from -20°C storage and thaw at 2°C to 8°C overnight to avoid potential precipitation.

CAUTION: DO NOT thaw frozen medium at 37°C. Please avoid repeated freezing/thawing.

For long-term liver spheroid culture (up to a few weeks), we recommend customers to supplement medium with desired antibiotics. If needed, filter the supplemented medium with 0.22 µM filter system.

Receiving and Unpacking

1. Unpacking the shipping package

- ▶ Set up the tissue culture hood, let the air flow at least 15 minutes before use.
- ▶ Open the taped box using scissors or box cutters, inspect the contents of the Corning HepGo Assay-ready 3D Liver Spheroid Kit package.

2. Unpacking the liver spheroid microplate package

- ▶ Remove the gel pack on top. Remove the temperature logger from the bubble bag, click stop on the temperature logger.
- ▶ Send the logger back to Corning for shipping temperature record using the return envelope included in the shipment.
- ▶ Remove the kit medium (100 mL bottle) from the bubble bag, keep warm in a 37°C water bath.
- ▶ Open the white mailer box; remove the plate package (wrapped in the bubble bag) from the white mailer box. Spray the microplate package with 70% ethanol.
- ▶ Keep the plate package face up, and transfer the microplate package to the TC culture hood.
- ▶ Keep the plate package face up, open the bubble bag, and take out the sterile plastic bag with the plate.
- ▶ Take the plate out of the sterile plastic bag.
- ▶ Open the lid of the plate, then remove the silicon gasket. **DO NOT remove the sterile sealing film at this step.**
- ▶ Put the lid back on the plate, centrifuge the plates at 100g for 2 minutes at room temperature.
- ▶ Check the liver spheroid plate under a microscope for spheroids.
- ▶ Wipe the plates with an alcohol wipe, and transfer back to the hood.
- ▶ Bring autoclaved stainless steel forceps or tweezers with serrated tips to the hood.
- ▶ Open the plate lid, hold the plate firmly with one hand. Starting from one corner, peel the sealer off gently with the other hand, avoiding sudden movement or jerking of the plate. (If the sealing film gets ripped by accident, use the autoclaved stainless-steel forceps or tweezers to remove any residual sealing film).

3. Medium Change

- ▶ Bring the pre-warmed medium to the hood; pour the needed amount of medium into a sterile 50 mL reservoir.
- ▶ Set aside another 50 mL reservoir for collection of the used medium.
- ▶ Using a multi-channel pipettor and sterile tips, place the pipet tip gently against the sidewall of the culture wells (refer to Figure 1). **Do not put the tip directly onto the bottom of the well to avoid losing spheroids during the medium change.**

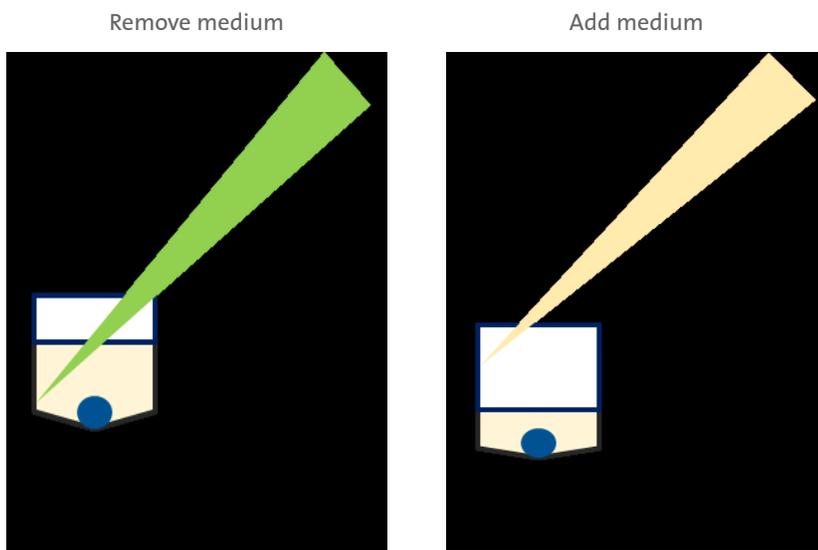


Figure 1. Adding and removing medium.

- ▶ Remove 200 μ L used medium from each well.
 - ▶ Add 100 μ L pre-warmed medium back to each well.
- NOTE:** Remove and add medium slowly to avoid removing the spheroid out of the well when performing the medium change.
- ▶ Using a microscope, check the morphology of the spheroid.
 - ▶ Transfer plates to a 37°C, 5% CO₂ incubator
 - ▶ Routine maintenance of the liver spheroids can be done by half medium change every other day.

NOTE: We recommend allowing the plates to recover overnight before starting assays.

Frequently Asked Questions

1. What happens if I thaw the Corning® HepGo™ Hepatocyte Culture Medium (Cat. No. 454670) at 37°C?

Thawing the frozen medium directly at 37°C may cause precipitation and therefore should be avoided. Thawing the frozen medium can be done in a 4°C fridge overnight before planned use. Once thawed, medium can be pre-warmed at 37°C before use.

2. How many cells are in each spheroid?

1,000 human hepatocytes were seeded per well on the 96-well microplate to grow liver spheroids.

3. What is the size of the PHH liver spheroids?

Size may vary depending on the donor PHH lot. Usually around 200 to 250 micron in diameter.

4. How is the viability of the PHH liver spheroids measured?

We routinely conduct ATP bioluminescent assay to assess the viability of individual PHH liver spheroids.

5. What other assays can be done with liver spheroids? Are there other applications available using the liver spheroids?

PHH liver spheroids have been used for *in vitro* hepatotoxicity assays with different end points (ATP, albumin secretion, image-based, etc.). For method development using PHH liver spheroids.

6. Instead of half medium change, can I do a more complete medium change?

There is the risk of aspirating and losing the spheroids during the medium change because these spheroids are in suspension, especially with manual operation. It is feasible to conduct a more complete medium change with an automated liquid handling system. Specific procedures need to be established using the parameters associated with the specific liquid handling equipment and Corning 96-well spheroid microplates.

7. Is it possible to order PHH liver spheroids from different donors?

Please contact your local Corning Sales Specialist for more detailed information.

8. How long can the liver spheroid culture last?

We have cultured the PHH liver spheroids up to 4 weeks with half medium change every other day.

9. What is in the Corning HepGo Hepatocyte Culture Medium?

Corning HepGo Hepatocyte Culture Medium is a William's E based serum-free, antibiotic-free medium.

10. How do I minimize the evaporation effects on the edge wells?

For long-term culture, medium evaporation especially in the edge wells needs to be taken into consideration. To minimize the effect, it is recommended to maintain at least 95% humidity in the incubator, and try to reduce the times of opening the incubator door if possible. Spheroid culture plates may be placed as close as possible to the water pan for humidity. Devices such as a secondary container with an additional water pan inside a TC incubator may help as well.

For more specific information on claims, visit www.corning.com/certificates.

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