

3D Spheroid Culture of Lonza NoSpin™ HepaRG® Hepatocarcinoma Cell Line for Use in Toxicity Assays

Instructions for use

Safety Statements

These products are not for human or animal *in vivo* or diagnostic use, including use as a diluent or as an excipient.

These products are for research use *only*.

WARNING: LONZA PRIMARY CELLS CONTAIN HUMAN SOURCE MATERIAL; TREAT AS POTENTIALLY INFECTIOUS. Each donor is tested and found non-reactive by an FDA-approved method for the presence of HIV-1, hepatitis B virus and hepatitis C virus. Where donor testing is not possible, cell products are tested for the presence of viral nucleic acid from HIV-1, hepatitis B virus, and hepatitis C virus. Testing cannot offer complete assurance that HIV-1, hepatitis B virus, and hepatitis C virus are absent. All human-sourced products should be handled at the biological safety level 2 to minimize exposure to potentially infectious products, as recommended in the CDC-NIH manual, [Biosafety in Microbiological and Biomedical Laboratories, 5th edition](#). If you require further information, please contact your site safety officer or Scientific Support.

Preparation of Reagents

All work should be performed in a laminar flow hood. Decontaminate the external surfaces of all supplement vials and the medium bottles with 70% ethanol or isopropanol.

1. Thawing and Plating Medium

- a. In a sterile 500 mL bottle, combine 1 mL 100X GlutaMax® (Gibco part no. 35-050-061) with 100 mL Williams E. Medium (without phenol red; Gibco A1217601 or equivalent).
- b. Add 1 tube (11.3 mL each) of thawed HepaRG® Thawing and Plating Additive (Lonza product no. MHTAP) to the above mixture.

- c. Total volume = 112.3 mL (scale all volumes based on number of vials of HepaRG® cells to be thawed).
- ##### 2. Induction Medium
- a. In a sterile 500 mL bottle, combine 1 mL 100X GlutaMax® (Gibco part no. 35-050-061) with 100 mL Williams E Medium (without phenol red; Gibco A1217601 or equivalent).
 - b. Using a pipette, transfer the entire contents (0.6 mL) of 1 tube of HepaRG® Serum-Free Induction Medium Supplement (Lonza part no. MHIND) to the above mixture.
 - c. Total volume = 101.6 mL (scale all volumes based on the number of wells to be plated).
 - d. Store at 4°C for up to 1 month.

HepaRG™ Thawing

NOTE: All work is to be performed in a laminar flow hood.

1. Warm the **Thawing and Plating Medium** in a 37°C water bath.
 2. Pipette 7.5 mL Thawing and Plating Medium into a sterile 40 mL round bottom polystyrene tube. Keep this tube at 37°C.
- NOTE:** Perform steps 3–7 within 4 minutes to thawing HepaRG® cells at room temperature as this can impact cell viability.
3. Remove cryopreserved HepaRG® cell vial (Lonza part no. NSHPRG) from liquid nitrogen.
 4. Wipe the cryovial with 70% ethanol or isopropanol before moving to the laminar flow hood. In the hood, briefly twist the cap a quarter turn to release pressure, and then re-tighten.
 5. Quickly transfer the vial to the 37°C water bath. Do not submerge and do not allow water to penetrate the cap!
 6. Agitate the vial back and forth in the water bath for 90 seconds until only a sliver of ice remains in the vial. Remove from the ice bath and wipe

the cryovial with 70% ethanol or isopropyl alcohol.

7. Using a pipette, carefully mix the 0.5 mL of cell suspension by gently pipetting up and down and then aseptically transfer the cell suspension into the 50 mL tube containing the 7.5 mL pre-warmed Thawing and Plating Medium.
8. To recover all cells, pipette approximately 1 mL of the cell suspension from the 40 mL tube back into the cryovial. Swirl and then transfer back to the 40 mL tube.

NOTE: The total volume in the 40 mL tube will equal 8 mL.

9. Reduce cell clusters by gently pipetting the suspension a few times. It is impossible to eliminate all clusters, but this will help.
10. Using Thawing and Plating Medium, adjust the volume of the cell suspension to obtain a density of 1×10^6 cells/mL.
 - a. Each lot of HepaRG[®] has its own guaranteed total population of viable cells, which is provided on the COA. Therefore, the amount of Thawing and Plating Medium required should be calculated based on the number of viable cells on the COA.
 - b. For example:
 - i. Lot # HNS1026 has a guaranteed yield of 9.2×10^6 cells per vial.
 - ii. To adjust the cell suspension, add an additional 1.2 mL of Thawing and Plating medium to the 8 mL of cell suspension for a final volume of 9.2 mL
 - iii. This brings the cell density to 1×10^6 cells/mL.
 - c. We suggest performing a cell count using 0.4 % trypan blue and a hemocytometer to validate this adjustment the first time that you use a given lot of HepaRG[®] cells.

HepaRG[®] Plating and Spheroid Formation

NOTE: All work is to be performed in a laminar flow hood.

1. Seed 20,000 cells/mL in Thawing and Plating Medium in an ultra-low attachment 96-well plate (100 μ L per well).
 - a. Combine 2 mL of 1×10^6 cells/mL cell with 98 mL of Thawing and Plating Medium.
 - b. Add 100 μ L of diluted cell suspension per well

NOTE: Do not spin! Homogenize cell suspension by gently pipetting up and down prior to plating each well.

2. Incubate plates at 37°C, 5% CO₂. Do not disturb.

3. Perform 50% medium changes with **Induction Medium** on days 5 and 6. Spheroids should be formed by day 7.

Suggested Use: Drug-Induced Liver Injury Toxicity Panel

Spheroids should be dosed with the compounds to be tested on day 7 as part of a 50% medium change. Spheroids should be re-dosed as part of 50% medium changes every 2–3 days until day 21.

Toxicity can be assessed by a variety of means. For example, using the Lonza ViaLight[®] Plus Cytotoxicity Bioassay Kit (measures cellular ATP as a measure of viability), spheroids should be harvested from each treatment on days 10, 14, and 21 to measure hepatotoxicity across acute and chronic timeframes.

Ordering Information

Catalog No.	Description	Size
NSHPRG	Cryopreserved NoSpin [™] HepaRG [®] cells	\geq 8 million cells
MHTAP	NoSpin [™] HepaRG [®] Thawing and Plating Additive	11.3 mL
MHIND	NoSpin [™] HepaRG [®] Serum-free Induction Additive	0.6 mL
LT07-321		10,000 test kit
LT07-121	Lonza ViaLight [®] Plus BioAssay Kit	1,000 test kit
LT07-221		500 test kit

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For more details: www.lonza.com/legal.