HepaRG™ Cells

Catalog Number HPRGC10

Pub. No. MAN0018571 **Rev.** B.0

WARNING! Read the Safety Data Sheets (SDSs) and follow the handling instructions. Wear appropriate protective eyewear, clothing, and gloves. Safety Data Sheets (SDSs) are available from **thermofisher.com/support**.

Product description

Gibco™ HepaRG™ cells have the unique properties of maintaining significant levels of hepatic cell functions, of being CYP450 inducible and supporting the complete replicative cycle of HBV.

Contents and storage

Contents	Amount	Storage
HepaRG™ Cells	≥10 × 10^6 viable cells/vial	Liquid nitrogen

Required materials not supplied

Unless otherwise indicated, all materials are available through **thermofisher.com**. MLS: Fisher Scientific (**fisherscientific.com**) or other major laboratory supplier.

Item	Source
Media (For use with 100 mL William's E Medium)	
HepaRG™ Thaw, Plate & General Purpose Medium Supplement	HPRG670
HepaRG™ Maintenance/Metabolism Medium Supplement	HPRG620
HepaRG™ Induction Medium Supplement	HPRG640
HepaRG™ Serum-free Induction Medium Supplement	HPRG650
Media (For use with 500 mL William's E Medium)	
HepaRG™ Thaw, Plate & General Purpose Medium Supplement (5X)	HPRG770
HepaRG™ Maintenance/Metabolism Medium Supplement (5X)	HPRG720
HepaRG™ Induction Medium Supplement (5X)	HPRG740
HepaRG™ Serum-free Induction Medium Supplement (5X)	HPRG750
Media and supplements	
William's E Medium (1X), liquid	12551032
GlutaMAX™ Supplement	35050061
William's E Medium, GlutaMAX™ Supplement	32551020
Coated cell culture supports	
Collagen I, Coated Plate 6-Well	A1142801
Collagen I, Coated Plate 24-Well	A1142802
Collagen I, Coated Plate 96-Well	A1142803

Item	Source
Equipment	
Water bath at +37°C	MLS
Laminar flow food	MLS
Pipet-aid, pipettes and micropipettes	MLS
Multichannel pipettes	MLS
Polystyrene round-bottom tubes (40 mL) and petri dishes (92 × 17 mm) or similar containers	MLS
Incubator at +37°C with 5% CO_2	MLS
Phase-contrast microscope	MLS
Material for cell count (cell counting chamber, coverslips, 0.05% Trypan Blue Solution)	MLS

Media preparation

Prepare HepaRG™ Thaw, Plate & General Purpose Medium

Thaw the HepaRG™ Thaw, Plate & General Purpose Medium Supplement (Cat. Nos. HPRG670 and HPRG770) by placing the bottle in a +37°C water bath until completely thawed.

- Add the thawed HepaRG™ Thaw, Plate & General Purpose Medium Supplement (Cat. No. HPRG670) to 100 mL of William's E Medium and 1 mL GlutaMAX™ Supplement, or 100 mL William's E Medium with GlutaMAX™ Supplement.
- Add the thawed HepaRG™ Thaw, Plate & General Purpose Medium Supplement (5X) (Cat. No. HPRG770) to 500 mL of William's E Medium and 5 mL GlutaMAX™ Supplement, or 500 mL William's E Medium with GlutaMAX™ Supplement.

The working media, HepaRG™ Thaw, Plate & General Purpose Medium, is now ready for use. Store at +4°C for a maximum of one month.

Prepare HepaRG™ Maintenance/Metabolism Medium

Thaw the HepaRG™ Maintenance/Metabolism Medium Supplement (Cat. Nos. HPRG620 and HPRG720) by placing the bottle in a +37°C water bath until completely thawed.

- Add the thawed HepaRG™ Maintenance/Metabolism Medium Supplement (Cat. No. HPRG620) to 100 mL of William's E Medium and 1 mL GlutaMAX™ Supplement, or 100 mL William's E Medium with GlutaMAX™ Supplement.
- Add the thawed HepaRG™ Maintenance/Metabolism Medium Supplement (5X) (Cat. No. HPRG720) to 500 mL of William's E Medium and 5 mL GlutaMAX™ Supplement, or 500 mL William's E Medium with GlutaMAX™ Supplement.

The working media, HepaRG™ Maintenance/Metabolism Medium, is now ready for use. Store at +4°C for a maximum of one month.

Prepare HepaRG™ Induction Medium Supplement

Thaw the HepaRG™ Induction Medium Supplement (Cat. Nos. HPRG640 and HPRG740) by placing the bottle in a +37°C water bath until completely thawed.

- Add the thawed HepaRG™ Induction Medium Supplement (Cat. No. HPRG640) to 100 mL of William's E Medium and 1 mL GlutaMAX™ Supplement, or 100 mL William's E Medium with GlutaMAX™ Supplement.
- Add the thawed HepaRG™ Induction Medium Supplement (5X) (Cat. No. HPRG740) to 500 mL of William's E Medium and 5 mL GlutaMAX™ Supplement, or 500 mL William's E Medium with GlutaMAX™ Supplement.

The working media, HepaRG™ Induction Medium Supplement, is now ready for use. Store at +4°C for a maximum of one month.

Prepare HepaRG™ Serum-free Induction Medium

Thaw the HepaRG™ Serum-free Induction Medium Supplement (Cat. Nos. HPRG650 and HPRG750) by placing the bottle in a +37°C water bath until completely thawed.

- Add the thawed HepaRG™ Serum-free Induction Medium Supplement (Cat. No. HPRG650) to 100 mL of William's E Medium and 1 mL GlutaMAX™ Supplement, or 100 mL William's E Medium with GlutaMAX™ Supplement.
- Add the thawed HepaRG™ Serum-free Induction Medium Supplement (5X) (Cat. No. HPRG750) to 500 mL of William's E Medium and 5 mL GlutaMAX™ Supplement, or 500 mL William's E Medium with GlutaMAX™ Supplement.

The working media, HepaRG™ Serum-free Induction Medium, is now ready for use. Store at +4°C for a maximum of one month.

Thaw cells

1. Pre-warm the HepaRG™ Thaw, Plate & General Purpose Medium in the +37°C water bath.

2. Pipet 9 mL (per cryovial of HepaRG™ cells to be used) of pre-warmed HepaRG™ Thaw, Plate & General Purpose Medium into a sterile 40-mL polystyrene round-bottom tube or similar container.

   Prepare an absorbent paper with 70% ethyl alcohol.

3. Remove the cryovial from the liquid nitrogen.

4. Under the laminar flow hood, briefly twist the cap a quarter turn (do not open the cryovial completely) to release the internal pressure, and then close it again.

5. Quickly transfer the cryovial to the water bath at +37°C.

   Do not submerge it completely, being careful not to allow water to penetrate into the cap.

6. While holding the tip of the cryovial, gently agitate the vial for about 2 minutes.

   Small ice crystal should remain when removed from the water bath.

7. Wipe the outside of the cryovial with 70% ethyl alcohol on an absorbent paper, and place the cryovial under the laminar flow hood.

8. Aseptically transfer the "semi"-thawed HepaRG™ cell suspension into the tube containing 9 mL of the pre-warmed HepaRG™ Thaw, Plate & General Purpose Medium (resulting in a 1:10 ratio of cell suspension to total volume).

9. Rinse out the cryovial once with approximately 1 mL of the HepaRG™ Thaw, Plate & General Purpose Medium and return the resulting suspension to the 40-mL tube.

10. Centrifuge the differentiated HepaRG™ cell suspension 3 min at 500 × *g* at room temperature.

    Do not utilize a traditional hepatocyte centrifugation protocol because HepaRG™ cells are smaller in size and need a longer and faster centrifugation.

11. Aspirate the supernatant. To avoid aspiration of cells, leave a little volume of medium on the pellet.

12. Gently resuspend the differentiated HepaRG™ cell pellet in 5 mL of HepaRG™ Thaw, Plate & General Purpose Medium.

    Do not try to dissociate the bigger clusters.

Determine cell viability and count cells

1. Transfer 900 µL Trypan Blue Solution (0.05% in DPBS 1X) in a 5-mL polystyrene round bottom tube.

2. Prepare a cell counting chamber (e.g., Nageotte chamber).

   Carefully clean the counting chamber the mirror-like polished surface with lens paper.

   Clean the coverslip.

   Place the coverslip over the counting surface before prior to putting on the cell suspension.

3. Gently homogenize the cell suspension by manual swirling.

4. Pipet approximately 100 µL of cell suspension between the mirror-like polished surface and the coverslip.

   The area under the coverslip fills by capillary action. Enough liquid should be introduced so that the mirrored surface is just covered.

5. Observe under a microscope and count living and dead cells on at least four rows distributed throughout the cell counting chamber.

   Living cells exclude the dye, dead cells take up the dye and appear blue. If the total number of cells is quite different from one row to another, count one or two more rows.

IMPORTANT! Thawed HepaRG™ cells can form clusters. It is necessary to count all the cells including those forming the clusters.

---

6. Determine the average number of viable cells and dead cells per row.

7. Determine percentage of cell viability.

$$\frac{Number\ of\ viable\ cells \times 100}{Number\ of\ viable\ cells + Number\ of\ dead\ cells}$$

8. Calculate the cell concentration in million cells / mL.

   Sample calculation with a Nageotte chamber:

$$(Number\ of\ viable\ cells\ per\ row \times 10)(dilution\ factor\ in\ Trypan\ Blue) \times 800\ (parameter\ relating\ to\ Nageotte\ cell) = M\ cell/mL$$

9. Calculate the total viable cell number:

$$\frac{(Cell\ concentration\ in\ million\ cells)}{mL \times (Total\ volume\ of\ cell\ suspension)} = Total\ number\ of\ cells$$

Metabolism studies: Use HepaRG™ cells in suspension

1. Thaw the cells in HepaRG™ Thaw, Plate & General Purpose Medium.
2. Incubate the cells with the test substrates according to your protocol.

Metabolism studies: Use HepaRG™ cells in monolayer

Seed HepaRG™ cells (monolayer for metabolism studies)

After thawing and the counting the HepaRG™ cells, and using the HepaRG™ Thaw, Plate & General Purpose Medium, seed the HepaRG™ cells into flat bottom multi-well plate(s) or flask(s) according to the table:

Table 1 Cryopreserved differentiated HepaRG™ cells

Cell culture support	Number of viable cells per well	Volume per well/flask	Cell concentration
25 cm^2 flask	5.2×10^6	5 mL	1.04×10^6/mL
6-well plate	2×10^6	2 mL	1×10^6/mL
12-well plate	0.8×10^6	1 mL	0.8×10^6/mL
24-well plate	0.48×10^6	0.5 mL	0.96×10^6/mL
48-well plate	0.16×10^6	0.2 mL	0.8×10^6/mL
96-well plate	0.072×10^6	0.1 mL	0.72×10^6/mL
384-well plate	0.024×10^6	0.04 mL	0.60×10^6/mL

Procedural guidelines to seed cells

- Pre-wet 96-well plate with 45 µL HepaRG™ Thaw, Plate & General Purpose Medium.
- If 96-well plate(s) are seeded partially, fill the wells surrounding those containing the cells with sterile water.
- To avoid "edge effect" (evaporation of medium from wells), fill outer rows with 50 µL water which effectively leaves 200 usable wells. For even distribution of cells in their wells, avoid causing air bubbles in the wells during cell seeding. This can be accomplished by first dispensing 20 µL medium, spinning the plate at 1500 × *g* for 5 minutes, and then adding 20 µL suspended cells at double the cell concentration shown above (so, at 1.2×10^6 cells/mL). Avoid having volumes <35 µL (0.035 mL) or >50 µL (0.05 mL) in any wells containing cells.
- Except for the 96- and 384-well plates, gently agitate the supports in a back-and-forth and side-to-side manner and visually control the homogeneity of the cell distribution.
- Place the plate(s) or flask(s) in the incubator at +37°C, 5% CO_2 and saturating humidity.

Cell maintenance for metabolism studies

Use the cells immediately after thawing, or following at least 3 days of culture. HepaRG™ cells keep a high level of CYP activities during the first 24 hours following thaw and plating, and these activities then decrease while the cells reconstitute the monolayer, then the activities return during the fourth day in culture, peaking at Day 8.

Use monolayer at Day 1, 4 hours after plating

Cells can be used for the metabolism studies according to your standard protocol with human hepatocytes.

Day	Hours after plating	Action
Day 1	4 hours	• Thaw and seed the cells using HepaRG™ Thaw, Plate & General Purpose Medium. • Four hours after plating, observe cell morphology under phase-contrast microscope and when possible, take photomicrographs. • Incubate the cells with the test substrates according to your protocol.

Use monolayer at Day 5, 96 hours after plating

At day 5 after thawing and culture: a cell monolayer can be observed with a hepatocyte-like cell organization in clusters and metabolic activities are slightly lower than activities detected from fresh cells.

Day	Day of week	Action
Day 1	Thursday	Thaw and seed the cells using HepaRG™ Thaw, Plate & General Purpose Medium.
Day 2 (24 hours)	Friday	Remove HepaRG™ Thaw, Plate & General Purpose Medium, and replace with the HepaRG™ Maintenance/Metabolism Medium.
Day 5 (96 hours)	Monday	Incubate the cells in monolayer with the test substrates according to your protocol.

Use monolayer at Day 8, 168 hours after plating

For optimal activity levels, HepaRG™ Maintenance/Metabolism Medium must have been renewed at Day 5 and Day 7.

At day 8 after thawing and culture: cells are organized in well-delineated trabeculae with many bright canaliculi-like structures and basal metabolic activities similar to fresh cells.

Day	Day of week	Action
Day 1	Thursday	Thaw and seed the cells using HepaRG™ Thaw, Plate & General Purpose Medium.
Day 2 (24 hours)	Friday	Remove HepaRG™ Thaw, Plate & General Purpose Medium, and replace with the HepaRG™ Maintenance/Metabolism Medium.
Day 5 (96 hours)	Monday	Renew the HepaRG™ Maintenance/Metabolism Medium.
Day 7 (144 hours)	Wednesday	Renew the HepaRG™ Maintenance/Metabolism Medium.
Day 8 (168 hours)	Thursday	Incubate the cells in monolayer with the test substrates according to your protocol.

Note: Cells can be used for the metabolism studies from Day 5 to Day 8 according to your standard protocol with human hepatocytes. They can also be kept in HepaRG™ Maintenance/Metabolism Medium for 1 additional week, provided that renewal of the HepaRG™ Maintenance/Metabolism Medium is performed every 2–3 days.

For 12- 24- 48- 96- 384-well plate(s)

1. Pre-warm the HepaRG™ Maintenance/Metabolism Medium in a sterile container (12 mL/24- or 12-well plate, 9.6 mL/48- or 96-well plate, 8 mL/384-well plate [assuming 200 usable wells], plus a little extra) at room temperature.
2. Transfer the pre-warmed HepaRG™ Maintenance/Metabolism Medium into a 92 × 17 mm Petri dish or similar flat-bottom container suitable for use with multichannel pipette.
3. Remove the lid from the multi-well plate.
4. Remove the existing medium from the wells.
5. Gently add the pre-warmed HepaRG™ Maintenance/Metabolism Medium to the sides of each well with a multichannel pipette (for volume per well, see Table 1).

   Do not add the medium directly onto the cells.
6. Control visually the medium level in the wells.
7. Put the lid back on the multi-well plate and place the plate(s) back in the +37°C incubator.

For 6 well plate(s):

1. Pre-warm the HepaRG™ Maintenance/Metabolism Medium in a sterile container (12 mL/6-well plate) at room temperature.
2. Remove the lid from the multi-well plate.
3. Remove the existing medium from the wells.
4. Gently add the pre-warmed HepaRG™ Maintenance/Metabolism Medium to the sides of each well with a pipette (2 mL per well).

   Do not add the medium directly onto the cells.
5. Control visually the medium level in the wells.
6. Put the lid back on the multi-well plate and place the plate(s) back in the +37°C incubator.

For 25 cm^2 flask(s)

1. Pre-warm the HepaRG™ Maintenance/Metabolism Medium at room temperature.
2. Remove the cap from the flask(s).
3. Aspirate the existing medium from the flask.
4. Transfer 5 mL of pre-warmed HepaRG™ Maintenance/Metabolism Medium into the 25 cm^2 flask.

   Take care not to pipette down the medium directly on the cells.
5. Replace the cap on the flask and place the flask(s) back in the +37°C incubator.

Induction studies: Use HepaRG™ cells in monolayer

For cell seeding, see "Seed HepaRG™ cells (monolayer for metabolism studies)" on page 4.

Culture and maintenance for induction study

1. Six hours after plating (see the suggested timeline), observe cell morphology under phase contrast microscope, and when possible, take photomicrographs.
2. Renew the HepaRG™ Thaw, Plate & General Purpose Medium (see "Cell maintenance for metabolism studies" on page 4).
3. At day 4, after 72 hours of culture, observe cell morphology under phase-contrast microscope, and when possible, take photomicrographs.
4. Cells can be used for induction studies; choose between media with:
   - **No serum**: HepaRG™ Serum-free Induction Medium
   - **Low level of serum**: HepaRG™ Induction Medium

5. Change from the HepaRG™ Thaw, Plate & General Purpose Medium to either the HepaRG™ Serum-free Induction Medium or HepaRG™ Induction Medium with the test articles.

6. Incubate the cells with the test articles for 48 hours.

7. Renew the medium with the test articles daily and always with the medium chosen at the beginning of the study (either HepaRG™ Serum-free Induction Medium or HepaRG™ Induction Medium).

   Note: Maximal fold induction of metabolic activity may be achieved with 72-hour treatment time, but published data indicate that 48 hours of treatment is sufficient to demonstrate significant induction of CYP1A2, CYP2B6, and CYP3A4 metabolic activity using prototypical inducers.

   For assessment of enzyme induction by measuring mRNA levels, 24-hour treatment time is frequently used, but 48 hours of treatment will ensure maximum induction (if any) occurs.

Suggested timeline for induction studies

Day	Day of week	Action
Day 1	Friday morning	Thaw and seed the cells using HepaRG™ Thaw, Plate & General Purpose Medium.
Day 1 (6 hours)	Friday afternoon (6 hours after plating)	Renew the HepaRG™ Thaw, Plate & General Purpose Medium.
Day 4 (72 hours)	Monday morning	1. Remove the HepaRG™ Thaw, Plate & General Purpose Medium, and replace with the HepaRG™ Induction Medium or HepaRG™ Serum-free Induction Medium. 2. Incubate the cells in monolayer with the test articles according to your study design. The renewal of the medium with the test articles should be performed daily until Wednesday.
Day 5 (96 hours)	Tuesday morning	Renew the HepaRG™ Induction Medium or HepaRG™ Serum-free Induction Medium with the test articles.
Day 6 (120 hours)	Wednesday	End of the incubation with the test articles Incubate the cells with the test substrates.

Uptake and transport studies: Use HepaRG™ cells in suspension

1. Thaw the cells in HepaRG™ Thaw, Plate & General Purpose Medium.

2. Incubate the cells with the test substrates according to your protocol for uptake and transport studies.

Toxicity studies: Use HepaRG™ cells in monolayer

For cell seeding, see "Seed HepaRG™ cells (monolayer for metabolism studies)" on page 4.

Culture and maintenance for toxicity study

1. One day after thawing, observe cell morphology under phase-contrast microscope, and when possible, take photomicrographs.

2. Renew the HepaRG™ Thaw, Plate & General Purpose Medium (see "Cell maintenance for metabolism studies" on page 4).

3. Maintain the HepaRG™ cells in HepaRG™ Thaw, Plate & General Purpose Medium until the use of cells at day 8.

4. Renew the HepaRG™ Thaw, Plate & General Purpose Medium, and incubate the cells in monolayer with the test articles according to your protocol.

Suggested timeline for toxicity studies

Day	Day of week	Action
Day1	Thursday	Use HepaRG™ Thaw, Plate & General Purpose Medium to thaw and seed the cells.
Day 2 (24 hours)	Friday	Renew HepaRG™ Thaw, Plate & General Purpose Medium.
Day 5 (96 hours)	Monday	Renew HepaRG™ Thaw, Plate & General Purpose Medium.
Day 7 (144 hours)	Wednesday	Renew HepaRG™ Thaw, Plate & General Purpose Medium.
Day 8 (168 hours)	Thursday	Remove HepaRG™ Thaw, Plate & General Purpose Medium and incubate the cells in monolayer with the test articles according to your protocols.

Cell morphology

- After 24 hours of culture, hepatocyte-like cells appear in small, differentiated colonies, individualized (Figure 1, 1).
- After 72–96 hours of culture, a restructuring of cell monolayer can be observed with a hepatocyte-like cells' organization in clusters (Figure 1, 2).
- 120–144 hours after plating, hepatocyte-like cells are organized in well-delineated trabeculae with many bright canaliculi-like structures (Figure 1, 3).

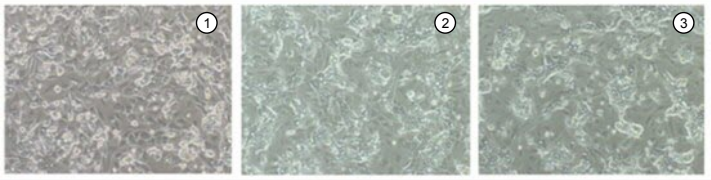

Figure 1 Cell morphology

Limited product warranty

Life Technologies Corporation and/or its affiliate(s) warrant their products as set forth in the Life Technologies' General Terms and Conditions of Sale at **www.thermofisher.com/us/en/home/global/terms-and-conditions.html**. If you have any questions, please contact Life Technologies at **www.thermofisher.com/support**.

Life Technologies Corporation | 7335 Executive Way | Frederick, MD 21704 | USA
For descriptions of symbols on product labels or product documents, go to **thermofisher.com/symbols-definition**.