gibco USER GUIDE

Cryopreserved 3D-Spheroid Qualified Human Hepatocytes

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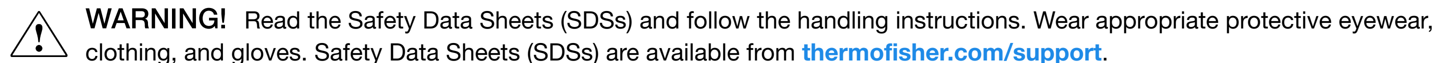

WARNING! Read the Safety Data Sheets (SDSs) and follow the handling instructions. Wear appropriate protective eyewear, clothing, and gloves. Safety Data Sheets (SDSs) are available from **thermofisher.com/support**.

IMPORTANT! Cryopreserved primary hepatocytes must be stored at all times in the vapor phase of liquid nitrogen, not submerged. The product should only be removed when it is to be thawed and cultured, which should occur immediately after removal from liquid nitrogen storage.

Product description

Gibco™ Cryopreserved 3D-Spheroid Qualified Human Hepatocytes are primary hepatocytes isolated from a single donor that can form 3D-spheroids using Nunclon™ Sphera™ Plates within 5-days of seeding. Cryopreserved 3D-Spheroid Qualified Human Hepatocytes are suitable for high throughput assays and require less cell input than standard 2D-cultures. 3D hepatocyte cultures have improved longevity compared to 2D hepatocyte cultures and can remain viable for up to 28 days. This increased longevity makes them suitable for testing low turnover compounds or chronic toxicity dosing. Every lot of cells is tested for Phase I enzymatic activity, viability post-thaw, and positive 3D-spheroid formation.

Contents and storage

Contents	Cat. No.	Amount	Storage
Cryopreserved 3D-Spheroid Qualified Human Hepatocytes	HMCPSQ	1 vial with 1.5 mL (>4 × 10^6 cells)	Store in liquid nitrogen vapor phase

Required materials not supplied

Unless otherwise indicated, all materials are available through **thermofisher.com**.

Catalog numbers that appear as links open the web pages for those products.

Item	Source
Nunclon™ Sphera™ 96U-well plate	174925
William's E Medium, no phenol red	A1217601
Hepatocyte Thaw Medium	CM7500
Primary Hepatocyte Thawing and Plating Supplements	CM3000
GlutaMAX™ Supplement	35050061
Penicillin-Streptomycin (10,000 U/mL)	15140122
Insulin-Transferrin-Selenium (ITS -G) (100X)	41400045
Dexamethasone	A13449

Product specifications

Description	Details
Form	Cryopreserved
Species	Human
Quantity	1 vial (1 mL)
Cell type	Hepatocytes
Donor source	1 donor
Product size	1.5 mL
Number of cells	>4 × 10^6 cells/vial

Procedural guidelines

Once thawed, cryopreserved hepatocytes must be used immediately and will not retain metabolic activities if refrozen.

IMPORTANT! Hepatocyte lots are qualified in Nunclon™ Sphera™ plates only and use of other plates can lead to variability in spheroid formation.

Prepare media

Plating Medium

To 500 mL William's E Medium add the following contents from the Primary Hepatocyte Thawing and Plating Supplements kit (Cat. No. CM3000):

- Entire contents of FBS bottle (25 mL)
- 50 µL Dexamethasone in DMSO
- Entire contents of Cocktail A (18 mL)

Store remaining Plating Medium at 4°C protected from light for up to one month.

Maintenance Medium

To 500 mL William's E Medium add:

- 5.0 µL Dexamethasone in DMSO
- 5 mL GlutaMAX™ Supplement
- 5 mL Penicillin-Streptomycin
- 5 mL Insulin-Transferrin-Selenium (ITS -G)

Store remaining Maintenance Medium (hereafter referred to as "Hepatic Spheroid Maintenance Medium") at 4°C protected from light for up to one month.

Thaw, centrifuge, and resuspend cells

1. Warm the following media to 37°C in a water bath:
    - Hepatocyte Thaw Medium
    - Plating medium

    IMPORTANT! Cryopreserved cells must be thawed quickly in a 37°C water bath.

2. Place the frozen vial in a 37°C water bath and rotate the vial intermittently until the content starts to thaw.
3. Promptly remove the vial from the water bath once a sliver of ice (avoid complete thawing) is left in the tube.
4. Decontaminate the external surface of the tube with 70% ethanol and transfer the contents of the cryovial to a 50-mL centrifuge tube containing pre-warmed Hepatocyte Thaw Medium.

    One tube of 45 mL Hepatocyte Thaw Medium is good for 1–5 vials of primary hepatocytes.

5. Centrifuge the tube at 100 × *g* for 10 minutes at room temperature after balancing the centrifuge appropriately.
6. After centrifugation, carefully aspirate the supernatant without disrupting the cell pellet.
7. Decontaminate the external surfaces of the plating media bottle with 70% ethanol and place in a biological safety cabinet.
8. Resuspend cell pellet with pre-warmed plating media by adding ~3 mL of media for each cell vial used and gently rocking the tube front and back repeatedly until no cell clump is observed.

Count and plate cells

1. Manually count live and dead cells using hemocytometer and adjust the volume with pre-warmed plating media so that a final live cell stock density of 1.5 × 10^6 cells/mL is achieved.
2. Remove 1 mL of cell stock from step 1 and transfer to a sterile bottle containing 99 mL of pre-warmed plating medium.

    This will yield a working cell suspension with a final density of 15,000 cells/mL. This volume can accommodate seeding up to 10 × 96-well plates.

3. Pre-wet a Thermo Scientific™ Nunclon™ Sphera™ 96-well plate with 100 µL of pre-warmed plating media per well.
4. Resuspend the cells once more by rocking front and back gently until all cell clumps are dissolved.
5. Add 100 µL of working cell suspension from step 2 to each well.

    This ensures 1500 cells are placed in each well and suspended in 200 µL of total media volume.

6. To seed other densities, take 1 mL of cell stock from step 1 and add pre-warmed plating media to obtain the desired density per volume.

7. After seeding, centrifuge the Nunclon™ Sphera™ plates containing cells at 200 × *g* for 2 minutes.

8. Place 96-well plate containing the seeded cells in a 37°C incubator with 5% CO_2 and allow incubation for 5 days undisturbed before changing media.

9. On day 5, carefully remove half of the media in each well (100 µL if total of 200 µL was used to seed cells) using a multichannel manual pipet or automated media/liquid handler.

   Pay close attention to media removal as not to disturb or discard the spheroids.

10. Add 100 µL of newly pre-warmed Hepatic Spheroid Maintenance Medium to each well and return the plates to incubator.

11. It is recommended that spheroids be cultured for at least 7 days before they are used for experiments. (5 days during spheroid formation in plating media + 2 days in hepatic spheroid maintenance medium)

    Longer culture time requires media changes every 48–72 hours. This method can maintain viable spheroids for up to 28 days.

12. For experimentation, prepare test article stock at 2X concentration. Perform media change (step 9) by removing half of the old media in each well and replace with same volume of new media containing 2X concentration of test compound. Incubate for appropriate time as per experimental design.

Related products

Unless otherwise indicated, all materials are available through **thermofisher.com**.

Catalog numbers that appear as links open the web pages for those products.

Item	Source
Wellwash™ Microplate Washer	5165000
Wellwash™ Versa Microplate Washer	5165010

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 | 3175 Staley Road | Grand Island, New York 14072 USA
For descriptions of symbols on product labels or product documents, go to thermofisher.com/symbols-definition.

The information in this guide is subject to change without notice.

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