Human iPSC-Derived Hepatic Organoids

Frozen hepatocyte-like cell-derived organoids generated from human induced pluripotent stem cell line, SCTi004-A

Catalog #200-1111 1 Unit

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Product Description

Human induced pluripotent stem cell (iPSC)-Derived Hepatic Organoids are derived and manufactured from Healthy Control Human iPSC Line, Male, SCTi004-A (Catalog #200-0769) that was first differentiated to hepatocyte-like cells using STEMdiff™ Hepatocyte Kit (Catalog #100-0520) and subsequently used to generate hepatic organoids using STEMdiff™ Hepatic Organoid Growth Medium (Catalog #100-1773).

Each unit is a cryovial of Human iPSC-Derived Hepatic Organoids that can be recovered, expanded for several passages, and scaled up using STEMdiff™ Hepatic Organoid Growth Medium. These organoids can be differentiated using STEMdiff™ Hepatic Organoid Differentiation Medium (Catalog #100-1774) to yield hepatic organoids that express mature hepatocyte markers such as ALB, CYP3A4, ASGR1, and CPS1 and exhibit mature hepatic functionality, including albumin secretion and CYP3A4 activity. Human iPSC-Derived Hepatic Organoids provide an expandable, three-dimensional, in vitro organotypic culture system for the study of hepatic development, drug-induced liver injury (DILI), and metabolism.

Cells used to generate this product were obtained using Institutional Review Board (IRB)-approved consent forms and protocols.

Stability and Storage

Product stable at -135°C or colder for 12 months from date of receipt. Thawed samples must be used immediately.

Organoid fragments are frozen in a cryopreservation medium containing dimethyl sulfoxide (DMSO)*.

* This product contains components dissolved in dimethyl sulfoxide (DMSO). DMSO is a strong solvent and skin penetrant, and can transport many substances through the skin. DMSO can also penetrate some protective glove materials including latex and silicone. Extra caution should be utilized when handling this product.

Precaution

Cell Screening: hiPSC master cell banks are screened for AAV2, BK virus, Epstein-Barr Virus, Hepatitis A, Hepatitis B, Hepatitis C, Herpes Simplex 1 and 2, Herpes Virus Type 6, Herpes Virus Type 7, Herpes Virus Type 8, HIV-1, HIV-2, HPV-16, HPV-18, Human Adenovirus, Human Cytomegalovirus, Human Foamy Virus, Human T-Lymphotropic Virus, John Cunningham Virus, LCMV, Parvovirus B19, Sarbecovirus (SARS Virus), Seoul Virus, Corynebacterium Bovis, and Mycoplasma (Human Comprehensive CLEAR Panel) by PCR. As testing cannot completely guarantee that the donor was virus-free, THIS PRODUCT SHOULD BE TREATED AS POTENTIALLY INFECTIOUS and only used following appropriate handling precautions such as those described in biological safety level 2.

Storage of frozen cell products in the vapor phase of a liquid nitrogen storage tank is recommended. Storage in the liquid phase can result in cross-contamination if the vial breaks or is not sealed properly. Storage in the liquid phase also increases the potential for liquid nitrogen to penetrate the vial and cause it to explode when removed from storage. Use of a face shield is required as a safety precaution when transferring cell products from one container to another. When handling this product, do not use sharps such as needles and syringes.

STEMCELL cannot guarantee the biological function or any other properties associated with performance of cell products in a researcher's individual assay or culture systems.

FOR IN VITRO RESEARCH USE ONLY. NOT APPROVED FOR DIAGNOSTIC, THERAPEUTIC, OR CLINICAL APPLICATIONS. NOT APPROVED FOR HUMAN OR VETERINARY USE IN VIVO.

Materials Required but Not Included

PRODUCT NAME	CATALOG #
24-well, tissue culture-treated plate OR 24-well, Organoid Culture Plates	e.g. 38017 OR 200-0561
Bovine serum albumin (BSA)	---
Conical tubes, 15 mL and 50 mL	e.g. 100-0092 and 100-0090
Corning® Matrigel® Growth Factor Reduced (GFR) Basement Membrane Matrix, Phenol Red-free, LDEV-free* (Matrigel®)	Corning 356231
D-PBS (Without Ca++ and Mg++)	37350
DMEM/F-12 with 15 mM HEPES	36254
Pipettor, 200 µL	e.g. 38059
Serological pipettes, 10mL and 25mL	e.g. 38004 and 38005
STEMdiff™ Hepatic Organoid Differentiation Medium	100-1774
STEMdiff™ Hepatic Organoid Growth Medium	100-1773

*We recommend using Matrigel® lots containing ≥ 8 mg/mL protein. Lower protein concentrations may reduce the long-term integrity of dome structures.

Preparation of Media and Cultureware

A. COMPLETE STEMDIFF™ HEPATIC ORGANOID GROWTH MEDIUM (OGM)

Use sterile technique to prepare complete STEMdiff™ Hepatic OGM (STEMdiff™ Hepatic Organoid Basal Medium + STEMdiff™ Hepatic Organoid Growth Supplement + antibiotics). The following example is for preparing 100 mL of complete medium. If preparing other volumes, adjust accordingly.

NOTE: 0.5 mL of complete STEMdiff™ Hepatic OGM is required per medium change for each well of a 24-well plate.

a. Thaw STEMdiff™ Hepatic Organoid Growth Supplement overnight at 2 - 8°C. Mix well.

   NOTE: If not using immediately, aliquot and store at -20°C. Do not exceed the shelf life of the supplement. Do not re-freeze aliquots after thawing.

b. Add 5 mL of STEMdiff™ Hepatic Organoid Growth Supplement to 95 mL of STEMdiff™ Hepatic Organoid Basal Medium.

c. Optional: Add antibiotics (e.g. final concentration 50 µg/mL gentamicin).

d. Mix well. Warm to room temperature (15 - 25°C) before use.

   NOTE: If not used immediately, store complete STEMdiff™ Hepatic OGM at 2 - 8°C for up to 2 weeks.

B. CULTUREWARE AND MATRIGEL®

1. One vial of Human iPSC-Derived Hepatic Organoids can be seeded into 3 wells of a 24-well tissue culture-treated plate or Organoid Culture Plate.
   - **If using a 24-well tissue culture-treated plate,** place it in a 37°C incubator for at least 1 hour.
   - **If using a 24-well Organoid Culture Plate,** pre-warming is not required; use plate at room temperature (15 - 25°C).
2. Thaw ~40 µL of Matrigel® for each dome to be seeded in a 24-well tissue culture-treated plate. If using an Organoid Culture Plate, thaw 60 µL of Matrigel® for each well.

   NOTE: Keep Matrigel® on ice when thawing and handling to prevent it from solidifying.

C. THAW/WASH MEDIUM

Use sterile technique to prepare Thaw/Wash Medium. The following example is for preparing 15 mL of Thaw/Wash Medium which is sufficient to thaw 3 cryovials of Human iPSC-Derived Hepatic Organoids. If preparing other volumes, adjust accordingly.

NOTE: If not used immediately, store Thaw/Wash Medium at 2 - 8°C for up to 1 month.

1. Combine 600 µL of 25% BSA solution prepared in water with 14.4 mL of DMEM/F-12 with 15 mM HEPES.
2. Mix well. Warm to room temperature (15 - 25°C) before use and store at room temperature for the duration of the protocol.

D. CONICAL TUBES AND CRYOVIALS

1. Add 2 mL of Thaw/Wash Medium (prepared in section C) to a 15 mL conical tube. Prepare one tube per cryovial to be thawed.
2. Retrieve cryovials containing Human iPSC-Derived Hepatic Organoids from liquid nitrogen immediately before use. Store on dry ice until ready to thaw.

Directions for Use

The following instructions are for thawing one cryovial of Human iPSC-Derived Hepatic Organoids and plating in 3 wells of a 24-well tissue culture-treated plate or a 24-well Organoid Culture Plate.

1. Working with one cryovial at a time, place the cryovial in a 37°C water bath to thaw for 2 - 2.5 minutes. Thawing is complete when the freezing medium becomes liquid. Proceed to the next steps immediately after the organoids are thawed.
2. Wipe the outside of the cryovial with 70% isopropanol and transfer to a biosafety cabinet.
3. Carefully uncap the cryovial and add 1 mL of Thaw/Wash Medium (see Preparation of Media and Cultureware, section C) to the cryovial. Using the same pipette tip, mix contents by pipetting up and down 4 times. Immediately transfer the contents to a 15 mL conical tube containing 2 mL of Thaw/Wash Medium (see Preparation of Media and Cultureware, section D).

   Optional: Pre-wet pipette tips with Thaw/Wash Medium before manipulating organoids to reduce adherence of organoid fragments to the pipette tip, which can reduce post-thaw yield and recovery.
4. Wash the inside of the cryovial and lid with 1 mL of Thaw/Wash Medium and add this volume to the 15 mL conical tube. Repeat this process with another 1 mL of Thaw/Wash Medium.

   NOTE: The 15 mL conical tube(s) containing thawed fragments can be stored on ice while thawing additional cryovials.
5. Centrifuge the 15 mL conical tube(s) at 300 x g for 5 minutes. Aspirate as much of the supernatant as possible without disturbing the pellet. Place the conical tube(s) on ice.
6. Place a pre-warmed 24-well tissue culture-treated plate or a room temperature 24-well Organoid Culture Plate in the biosafety cabinet (see Preparation of Media and Cultureware, section B).
7. Process one tube/pellet at a time, as described below. Work quickly to ensure Matrigel® does not solidify. Pipette tips can be cooled when working with Matrigel® to help minimize premature solidifying.

   NOTE: The 8 wells in the center of a 24-well tissue culture-treated plate are the most suitable for domes since their surfaces are the most even. Wells at the edges of the plate are often slightly slanted resulting in domes touching the wall of the well and flattening out.

   a. Using a pipettor with a 200 µL pipette tip, resuspend pellets as follows for each cryovial of Human iPSC-Derived Hepatic Organoids to be seeded:
      - Add 90 µL of thawed Matrigel® (i.e. 30 µL for each dome to be seeded) on top of the pellet, if using a 24-well tissue culture-treated plate.
      - Add 150 µL of thawed Matrigel® (i.e. 50 µL for each layer to be seeded) on top of the pellet, if using a 24-well Organoid Culture Plate.

   b. Gently mix the fragment-Matrigel® suspension by pipetting up and down 5 - 8 times without generating bubbles.

   c. Seed domes/layers, dispensing only to the first stop of the pipettor to avoid generating bubbles.
      - If using a 24-well tissue culture-treated plate, set the pipettor volume to 30 µL. To form domes, transfer 30 µL of fragment-Matrigel® suspension to the center of each of 3 wells. While dispensing, gradually move the pipette tip upward so that the fragments are evenly distributed throughout the dome.
      - If using an Organoid Culture Plate, set the pipettor volume to 50 µL. To seed matrix layers, transfer 50 µL of fragment-Matrigel® suspension to the center of the well, using the pipette tip to drag the suspension around the edges if required to fully fill any gaps.
8. Repeat step 7 for the remaining pellets/tubes.
9. Place the lid on the culture plate. Carefully place the plate in an incubator at 37°C and 5% CO_2 for 10 minutes to let Matrigel® solidify.
10. Remove the plate from the incubator and place in the biosafety cabinet.
11. Without disturbing the matrix, carefully add 500 µL of room temperature (15 - 25°C) complete STEMdiff™ Hepatic OGM (see Preparation of Media and Cultureware, section A) against the side of each well containing a dome/layer. Do not pipette directly onto the domes/layers.
12. Add room temperature sterile D-PBS (Without Ca++ and Mg++) to any unused wells. Place the lid on the culture plate. Incubate the plate at 37°C and 5% CO_2.
13. Perform a full-medium change every 2 - 3 days by carefully aspirating the medium and adding 500 µL of fresh room temperature complete STEMdiff™ Hepatic OGM to each well containing a dome/layer.

    NOTE: If Matrigel® domes/layers are loose, remove 400 µL of medium from the well, then add 400 µL of fresh medium. To avoid weekend medium changes, perform medium changes on Mondays, Wednesdays, and Fridays.

    NOTE: To monitor organoid growth, take images of the same field of view every 2 - 3 days until they are ready to be passaged.
14. Passage organoids before the lumen turns dark and the organoids collapse, usually every 6 - 10 days, as described in section 4.2 of Technical Manual: Establishment, Growth, and Differentiation of Human Pluripotent Stem Cell-Derived Hepatic Organoids Using STEMdiff™ Hepatic Organoid Media (Document #10000031146).

15. For use in downstream assays requiring organoids that exhibit mature hepatic phenotypes, differentiate hepatic organoids using STEMdiff™ Hepatic Organoid Differentiation Medium, as described in section 5.0 of Technical Manual: Establishment, Growth, and Differentiation of Human Pluripotent Stem Cell-Derived Hepatic Organoids Using STEMdiff™ Hepatic Organoid Media (Documen #10000031146).

    NOTE: Before setting up organoids for differentiation, expand Human iPSC-Derived Hepatic Organoids in complete STEMdiff™ Hepatic OGM for at least one passage to ensure typical organoid growth characteristics are restored post thaw.

    NOTE: Hepatic organoids can be expanded for several passages. For optimal maturation outcomes, differentiate organoids expanded for up to 10 passages post-thaw.

Related Products

For related products and resources, including specialized cell culture media, posters, and protocols, visit www.stemcell.com/organoid-workflows or www.stemcell.com/organoids-in-drug-discovery.