Mouse Hepatic Organoids

Cryopreserved mouse hepatic progenitor organoids for establishment of organoid cultures

Catalog #70932 2 culture wells

STEMCELL™ TECHNOLOGIES
Scientists Helping Scientists™ | WWW.STEMCELL.COM
TOLL FREE PHONE 1 800 667 0322 • PHONE +1 604 877 0713
INFO@STEMCELL.COM • TECHSUPPORT@STEMCELL.COM
FOR GLOBAL CONTACT DETAILS VISIT OUR WEBSITE

Product Description

Cryopreserved Mouse Hepatic Organoids provide a convenient way to establish or standardize hepatic organoid cultures in the laboratory. Each vial contains mouse hepatic progenitor organoids (derived from the liver of a C57BL/6 mouse) that were cultured in HepatiCult™ Organoid Growth Medium (Mouse; Catalog #06030) and cryopreserved in CryoStor® CS10 (Catalog #07930). Using cryopreserved Mouse Hepatic Organoids enables establishment of hepatic organoid cultures without the need to isolate hepatic ducts from primary tissue, eliminating the need for access to fresh mouse tissue. The organoids can be passaged and expanded using HepatiCult™ Organoid Growth Medium (Mouse) and refrozen in CryoStor® CS10. Mouse hepatic progenitor organoid cultures can be used for research in a variety of fields, including liver biology and disease modeling, cancer, and toxicity assay development.

Properties

Storage: Store at -135°C or colder.

Shelf Life: Stable for 3 years from date of manufacture (MFG) on label.

Contains:
- Frozen mouse hepatic organoid segments
- CryoStor® CS10

Materials Required But Not Included

PRODUCT NAME	CATALOG #
HepatiCult™ Organoid Growth Medium (Mouse)	06030
DMEM/F-12 with 15 mM HEPES	36254
Bovine serum albumin (BSA)	---
Corning® Matrigel® Growth Factor Reduced (GFR) Basement Membrane Matrix, Phenol Red-Free, LDEV-Free*	Corning 356231*
Costar® 24-Well Flat-Bottom Plate, Tissue Culture-Treated	38017
Falcon® Conical Tubes, 15 mL	38009

*We recommend using Corning® Matrigel® lots containing ≥ 8 mg/mL protein. Lower protein concentrations may affect organoid growth.

Directions for Use

The following instructions are for preparing one cryovial of organoids for plating in 2 wells of a 24-well tissue culture-treated plate. Use sterile techniques throughout the protocol.

NOTE: Pre-wet pipette tips with DMEM/F-12 with 15 mM HEPES + 1% BSA (prepared in step 4) before manipulating organoids. This prevents tissue from sticking to the wall of the pipette tip, which significantly decreases organoid yield.

SETUP

1. Place a 24-well tissue culture-treated plate in a 37°C incubator for at least 1 hour. Place a box of sterile 200 µL pipette tips at 2 - 8°C.
2. Thaw ~75 µL of Corning® Matrigel® on ice.

   NOTE: Keep Corning® Matrigel® on ice when thawing and handling to prevent it from gelling.
3. Prepare 1.6 mL of HepatiCult™ Organoid Growth Medium (refer to the Product Information Sheet [PIS] for HepatiCult™). Warm to room temperature (15 - 25°C).
4. For pre-wetting pipette tips, prepare DMEM/F-12 with 15 mM HEPES + 1% BSA (DMEM + BSA) as follows:
   a. Add 2 mL of sterile 25% BSA to 48 mL of DMEM/F-12 with 15 mM HEPES. Mix thoroughly. Store at room temperature (15 - 25°C) for the duration of the protocol.
   b. Store the remaining DMEM + BSA at 2 - 8°C for up to 1 month.

5. To a 15 mL conical tube, add 2 mL of DMEM + BSA (prepared in step 4).

   NOTE: Transfer cells to this tube immediately after thawing (steps 6 - 8) to avoid a significant reduction in viability.

THAWING ORGANOIDS

6. Place the cryovial of organoids in a 37°C water bath to thaw for 2 - 2.5 minutes. Thawing is complete when the freezing medium becomes liquid. Perform steps 7 - 8 immediately after cells are thawed.

   NOTE: Warming the frozen organoids for too long may affect the growth of the organoids in culture. Once thawed, do not re-freeze.

7. Wipe the outside of the cryovial with 70% ethanol or isopropanol before opening.
8. Using a 1 mL pipettor, add 1 mL of DMEM + BSA to the cryovial. Mix the contents by pipetting up and down 4 times. Immediately transfer the contents of the cryovial to the tube prepared in step 5.
9. Wash the inside of the cryovial and inside of the lid with 2 x 1 mL of DMEM + BSA. Add the washes to the organoid suspension from step 8.
10. Centrifuge the organoid suspension at 290 x *g* for 5 minutes. If there are bubbles on the surface, aspirate these first, then aspirate the remainder of the supernatant without disturbing the pellet, leaving ~5 - 10 µL in the tube. Place the tube on ice.

CULTURING ORGANOIDS IN MATRIGEL® DOMES

11. Remove the 24-well plate from the incubator and 200 µL pipette tips from the fridge and place in a biosafety cabinet.
12. Process the pellet as described below. Work quickly after adding Matrigel® to the pellet to ensure the Matrigel® does not solidify.

    NOTE: The 8 wells in the center of a 24-well 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 pipette with a cooled, pre-wetted 200 µL tip, add 62 µL of cold Matrigel® to the pellet. Mix by pipetting up and down 5 - 8 times.

    b. Set the pipette to 30 µL. Add 30 µL of organoid/Matrigel® suspension to each of 2 wells of the warm 24-well plate such that it forms a dome in the middle of the well. Avoid generating bubbles on top of the dome.

13. 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 domes solidify.
14. Remove the plate from the incubator and place in the biosafety cabinet.
15. Without disturbing the domes, carefully add 750 µL of HepatiCult™ Organoid Growth Medium against the side of each well containing a dome. Do not pipette directly onto the domes.
16. Add sterile PBS to any unused wells. Place the lid on the culture plate.
17. Capture one 2X image per well using a brightfield microscope (Day 0 images). Incubate at 37°C and 5% CO_2.

    NOTE: To monitor organoid growth, take photos of the same field of view within each dome every 2 - 3 days until they are passaged.

18. Perform a full-medium change every 2 - 3 days for up to 1 week by carefully aspirating the medium and adding 750 µL of fresh HepatiCult™ Organoid Growth Medium at room temperature (15 - 25°C). Organoids should be ready for passaging after 4 - 7 days.

    NOTE: If Matrigel® domes are loose, change medium by removing 250 µL of medium from the well, then add 500 µL of fresh medium.

    NOTE: To avoid weekend medium changes, perform medium changes on Mondays, Wednesdays, and Fridays.

    NOTE: Organoid recovery may be variable. Passage organoids 1 - 2 times before cryopreservation or downstream experiments to ensure typical organoid growth characteristics are restored. For a passaging protocol, refer to the PIS for HepatiCult™. For additional passaging and cryopreservation protocols, refer to the Technical Bulletin: Mouse Hepatic Progenitor Organoid Culture: Supplementary Protocols (Document #27087). Documents are available at www.stemcell.com or contact us to request a copy.