Mouse Pancreatic Organoids

Cryopreserved mouse pancreatic exocrine organoids for establishment of organoid cultures

Catalog #70933 200 Organoids

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 Pancreatic Organoids provide a convenient way to establish or standardize pancreatic organoid cultures in the laboratory. Each vial contains fragments of mouse pancreatic exocrine organoids sufficient to establish two wells of organoid cultures. The organoids are derived from the pancreas of C57BL/6 mice, cultured in PancreaCult™ Organoid Growth Medium (Mouse; Catalog #06040) and cryopreserved in CryoStor® CS10 (Catalog #07930). Using cryopreserved Mouse Pancreatic Organoids enables establishment of pancreatic organoid cultures without the need to isolate pancreatic ducts from primary tissue, eliminating the need for access to fresh mouse tissue. The organoids can be passaged and expanded using PancreaCult™ Organoid Growth Medium (Mouse) and cryopreserved in CryoStor® CS10. Mouse pancreatic exocrine organoid cultures can be used for research in a variety of fields, including epithelial cell biology, cancer, and metabolism.

Properties

Storage: Store at -135°C or colder.

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

Contains:
- Frozen mouse pancreatic exocrine organoid segments
- CryoStor® CS10

Materials Required But Not Included

PRODUCT NAME	CATALOG #
PancreaCult™ Organoid Growth Medium (Mouse)	06040
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 5 mL of PancreaCult™ Organoid Growth Medium (refer to the Product Information Sheet [PIS] for PancreaCult™). 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.

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. Dispense only to the first stop of the pipettor to 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 PancreaCult™ 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 PancreaCult™ 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.
19. Passage organoids 1 - 2 times before cryopreservation or downstream experiments; for a passaging protocol, refer to the PIS for PancreaCult™, available at www.stemcell.com or contact us to request a copy. For the first passage, pool the material from both domes plated in Step 12b (as per PancreaCult™ PIS, section B step 8); thereafter wells can be passaged individually if desired.

    NOTE: For additional passaging and cryopreservation protocols, refer to the Technical Bulletin: Mouse Pancreatic Exocrine Organoid Culture: Supplementary Protocols (Document #27088), available at www.stemcell.com or contact us to request a copy.