# PancreaCult™ Organoid Growth Medium (Mouse)

**Cell culture medium for establishment and maintenance of mouse pancreatic exocrine organoids**

Catalog #06040 1 Kit

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## Product Description

PancreaCult™ Organoid Growth Medium (Mouse) is a serum-free, defined cell culture medium for establishment and maintenance of mouse pancreatic exocrine organoids. These organoids, or "mini-pancreata", provide an in vitro organotypic culture system for studying pancreatic cell biology, disease, and cancer. Organoids grown in PancreaCult™ feature an epithelium expressing genes marking pancreatic stem cells (LGR5), progenitor cells (PDX1, SOX9), and ductal cells (CAR2, MUC1, KRT19). Pancreatic organoids can be passaged every 3 - 6 days for long-term maintenance and can also be cryopreserved.

PancreaCult™ supports mouse pancreatic organoid culture either embedded in Corning® Matrigel® domes or in a dilute Matrigel® suspension. Organoid culture enables convenient in vitro characterization of the pancreatic epithelium in a physiologically relevant system and reduces the need for animal use.

Should you intend to use this product for commercial purposes, please contact HUB at www.huborganoids.nl for a commercial use license or for clarification in relation to HUB licensing.

## Product Information

The following components are sold as a kit (Catalog #06040) and are not available for individual sale.

| COMPONENT NAME | COMPONENT # | SIZE | STORAGE | SHELF LIFE |
|---|---|---|---|---|
| PancreaCult™ OGM Mouse Basal Medium | 06041 | 95 mL | Store at 2 - 8°C. | Stable for 2 years from date of manufacture (MFG) on label. |
| PancreaCult™ OGM Mouse Supplement* | 06042 | 5 mL | Store at -20°C. | Stable for 2 years from date of manufacture (MFG) on label. |

*This component contains material derived from human plasma. Donors have been tested and found negative for HIV-1 and -2, hepatitis B, and hepatitis C prior to donation. However, this product should be considered potentially infectious and treated in accordance with universal handling precautions.

## Materials Required but Not Included

| PRODUCT NAME | CATALOG # |
|---|---|
| 70 µm Reversible Strainer, Large | 27260 |
| 100 mm Dish, Non-Treated | 38045 |
| Advanced DMEM/F-12 | Thermo Fisher 12634010 |
| Anti-Adherence Rinsing Solution | 07010 |
| Antibiotics (e.g. gentamicin) | --- |
| Collagenase Type IV (1 mg/mL) | 07909 |
| Corning® Matrigel® Growth Factor Reduced (GFR) Basement Membrane Matrix, Phenol Red-Free, LDEV-Free* | Corning 356231* |
| Costar® 6-Well Flat-Bottom Plate, Tissue Culture-Treated | 38015 |
| Costar® 24-Well Flat-Bottom Plate, Tissue Culture-Treated | 38017 |
| D-PBS (Without Ca++ and Mg++) | 37350 |
| Dispase (1 U/mL) | 07923 |
| DMEM/F-12 with 15 mM HEPES | 36254 |
| DNase I Solution (1 mg/mL) | 07900 |
| Falcon® Conical Tubes | 38009 (15 mL) and 38010 (50 mL) |
| Falcon® Serological Pipettes | 38004 (10 mL) and 38005 (25 mL) |

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

# Preparation of Reagents and Materials

### PancreaCult™ Organoid Growth Medium (Mouse)

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

1. Thaw the Supplement at 2 - 8°C overnight. Mix thoroughly.

   NOTE: Once thawed, use immediately or aliquot and store at -20°C. Do not exceed the shelf life of the supplement. After thawing the aliquots, use immediately. Do not re-freeze.
2. Add 5 mL of Supplement to 95 mL of Basal Medium.
3. Add antibiotics (e.g. 50 µg/mL gentamicin). Mix thoroughly. Warm to room temperature (15 - 25°C) before use.

   NOTE: If not used immediately, store PancreaCult™ Organoid Growth Medium at 2 - 8°C for up to 1 month.

### Tissue Dissociation Cocktail

1. Prepare 30 mL of Tissue Dissociation Cocktail (sufficient for one mouse pancreas) by combining the following:
   - 3.75 mL Collagenase Type IV
   - 3.75 mL Dispase
   - 22.2 mL DMEM/F-12 with 15 mM HEPES
   - 300 µL DNase I Solution (1 mg/mL)

   Mix thoroughly.

   NOTE: If not used immediately, store at 2 - 8°C for up to 1 month.
2. Warm to room temperature (15 - 25°C) before use.

### Pre-Wetting Conical Tubes and Serological Pipettes

Conical tubes and serological pipettes that come in contact with pancreatic tissue should be pre-wetted, as pancreatic tissue frequently adheres to their surfaces, significantly reducing organoid yield. Pre-wet tubes and pipettes on the day of the experiment.

For each pancreas being processed, pre-wet **12 x 15 mL conical tubes and 3 x 50 mL conical tubes** as follows:

1. Add 5 mL of Anti-Adherence Rinsing Solution to a 15 mL conical tube. Swirl to coat the tube.
2. Transfer the entire volume to a second 15 mL conical tube. Repeat until all 15 mL conical tubes have been coated.
3. Aspirate any remaining Rinsing Solution from the coated tubes.
4. Repeat steps 1 - 2 using Advanced DMEM/F-12 instead of Rinsing Solution. Aspirate any remaining Advanced DMEM/F-12 from coated 15 mL conical tubes.
5. To pre-wet 1 x 50 mL conical tube, use 30 mL Anti-Adherence Rinsing Solution and 30 mL Advanced DMEM/F-12 and follow steps 1 - 4.
6. Cap all coated tubes tightly and store at room temperature (15 - 25°C) until required.

Pre-wet **serological pipettes** as follows:

1. Add 25 mL of Anti-Adherence Rinsing Solution to a 50 mL conical tube. Add 25 mL of Advanced DMEM/F-12 to a second 50 mL conical tube. Place aliquots at room temperature (15 - 25°C).
2. Immediately before use, coat serological pipettes with aliquoted Anti-Adherence Rinsing Solution, followed by Advanced DMEM/F-12.

# Directions for Use

Please read the entire protocol before proceeding. Use sterile technique when performing the following protocols:

A. Organoid Initiation

B. Passaging Mouse Pancreatic Exocrine Organoids

### A. ORGANOID INITIATION

The following protocol is for initiating pancreatic exocrine organoids using pancreatic ducts from one mouse, and culturing in Matrigel® domes. For protocols using single cells and for culture in a dilute Matrigel® suspension, 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.

### SET-UP

1. Place 2 x 24-well plates 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 450 - 500 µL of Corning® Matrigel® on ice.

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

3. Prepare PancreaCult™ Organoid Growth Medium and Tissue Dissociation Cocktail. Warm to room temperature (15 - 25°C).
4. Add 30 mL of DMEM/F-12 with 15 mM HEPES to each of 2 x 100 mm dishes and place on ice. Place 2 x 50 mL pre-wetted conical tubes on ice.

## ISOLATION OF PANCREATIC DUCTS

5. Sacrifice mouse according to approved institutional guidelines. Keep mouse on ice until dissection. Within 2 hours of sacrifice, harvest the mouse pancreas and transfer it to a dish containing cold DMEM/F-12 (prepared in step 4).
6. Swirl pancreas in dish to wash. Use forceps to transfer pancreas to the second dish containing cold DMEM/F-12. With pancreas submerged in DMEM/F-12, cut up pancreas into small (~3 mm) pieces.

*For the remainder of the protocol, **pre-wet serological pipettes** as indicated in Preparation of Reagents and Materials.*

7. Using a 25 mL serological pipette, transfer pancreas pieces and DMEM/F-12 into a 50 mL tube on ice (prepared in step 4).
8. Let pancreas pieces settle by gravity on ice for 2 minutes. Aspirate and discard supernatant.
9. Add 5 mL of room temperature Tissue Dissociation Cocktail (see Preparation section) to pancreas pieces. Incubate tube in a 37°C water bath for 20 minutes.
10. Remove tube from water bath. Using a 10 mL pre-wetted serological pipette, vigorously pipette pancreas pieces up and down 7 times with medium-high force.
11. Let pancreas pieces settle by gravity for 1 minute.
12. Using a 10 mL serological pipette, remove and discard the supernatant (including digested cells suspended in it).

    NOTE: The digestion supernatant is only discarded in this first digestion cycle. In the remaining digestion cycles, retain the supernatant as indicated.
13. Repeat steps 9 - 11 (digestion cycle #2). Using a pre-wetted 10 mL serological pipette, collect supernatant and add to a new cooled pre-wetted 50 mL conical tube (prepared in step 4). Keep on ice.
14. Repeat steps 9 - 11 four more times (digestion cycles #3 - 6), collecting supernatant into the 50 mL tube on ice (from step 13) after each digestion.

    NOTE: Pancreatic ducts will be released into the supernatant with each successive digestion cycle. The pooled supernatant volume from digestion cycles #2 - 6 will be ~25 mL.
15. Attach a 70 µm cell strainer to a new 50 mL conical tube. Pre-wet the strainer with 5 mL of Anti-Adherence Rinsing Solution, followed by 5 mL of Advanced DMEM/F-12.
16. Using a pre-wetted 25 mL serological pipette, pass the pooled supernatant (from step 14) through the strainer. Discard the flow-through.
17. Reverse the strainer onto a pre-wetted 50 mL conical tube (see Preparation section). Using a 10 mL serological pipette, carefully add 2 x 12 mL of cold Advanced DMEM/F-12 to the reversed strainer to wash tissue fragments and ducts into the tube.

    NOTE: Ensure the tip of the serological pipette touches the surface of the strainer while the entire bottom surface is thoroughly rinsed. If tissue fragments still remain on the surface, use a serological pipette to gently scrape them off and transfer to the tube.
18. Using a pre-wetted 10 mL serological pipette, pipette the tissue fragments and ducts (collected in step 17) up and down 3 - 5 times to create an even suspension. Immediately transfer equal volumes into each of 12 x 15 mL pre-wetted conical tubes (see Preparation section).
19. Centrifuge the 12 tubes at 290 x *g* for 5 minutes. Aspirate as much of the supernatant as possible without disturbing the pellets, leaving ~5 - 10 µL in the tube (the pellet is often not visible). Place tubes on ice.

## CULTURE IN MATRIGEL® DOMES

20. Remove the first 24-well plate from the incubator and 200 µL pipette tips from the fridge and place in a biosafety cabinet.
21. Process one tube/pellet at a time, as described below. Work quickly 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 pipettor with a cooled 200 µL pipette tip, add 35 µL of thawed Matrigel® on top of the pellet. Without generating bubbles, gently mix the duct-Matrigel® suspension by pipetting up and down 5 - 8 times, going to only the first stop of the pipettor.
    b. Set the pipettor volume to 50 µL. Add the entire suspension to the center of 1 well of the 24-well plate to form a dome. While dispensing, gradually move the pipette tip upwards so that the ducts are evenly distributed throughout the dome. Dispense only to the first stop of the pipettor to avoid generating bubbles on top of the dome.
22. Repeat step 21 for an additional 7 pellets.
23. Remove the second 24-well plate from the incubator and repeat step 21 for the remaining 4 pellets.
24. Place a lid on each culture plate. Carefully place the plates in an incubator at 37°C and 5% $CO_2$ for 10 minutes to let domes solidify.

25. Remove the plates from the incubator and place in the biosafety cabinet. Without disturbing the domes, carefully add 750 µL of room temperature (15 - 25°C) PancreaCult™ Organoid Growth Medium against the side of each well containing a dome. Do not pipette directly onto the domes.
26. Add sterile PBS to any unused wells. Place the lid on each culture plate.
27. Capture one 2X image per dome using a brightfield microscope (Day 0 images). Incubate the plates at 37°C and 5% $CO_2$.

    NOTE: To monitor organoid growth, take photos of the same field of view every 2 - 3 days until they are passaged.
28. 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.

    NOTE: If Matrigel® domes are loose, remove 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.
29. Monitor organoids daily. They should be passaged before the lumen turns dark and collapses. This usually occurs at Day 3 - 6. Proceed to section B when organoids are ready for passage.

    NOTE: If there are no organoids or very few, small organoids in one well, proceed with section B steps 1 - 9. Centrifuge the fragment suspension at 290 x *g* for 5 minutes. Aspirate as much of the supernatant as possible without disturbing the pellet, leaving ~5 - 10 µL in the tube (the pellet is often not visible). Place tube on ice. Proceed with passaging into a single dome (section A steps 20 - 28).

## B. PASSAGING MOUSE PANCREATIC EXOCRINE ORGANOIDS

The following protocol is for passaging pancreatic exocrine organoids via fragment number followed by culture in Matrigel® domes. For single-cell passaging and for culture in a dilute Matrigel® suspension, 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.

1. Place a 24-well 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 Matrigel® on ice (~40 µL/well to be passaged). Place Advanced DMEM/F-12 on ice.
3. Prepare PancreaCult™ Organoid Growth Medium and bring to room temperature (15 - 25°C).
4. Check that the Matrigel® domes to be passaged are intact. If the dome is intact, proceed to step 5. If the dome is loose, add cold Advanced DMEM/F-12 to top up the total volume in the well to 1 mL and let sit for 1 minute; proceed to step 7.
5. Without touching the dome, aspirate and discard the medium in each well to be passaged.
6. Using a 1 mL pipettor, forcefully add 1 mL of cold Advanced DMEM/F-12 to the center of each dome and let sit for 1 minute.
7. Vigorously pipette the total volume up and down 15 times, taking care not to generate bubbles.

    NOTE: This results in mechanical breakdown of organoids and Matrigel® into smaller fragments of 30 - 100 µm. Check fragment sizes using a light microscope; if most fragments are larger than 100 µm, triturate until they are ≤ 100 µm.
8. If passaging multiple wells, pool the contents of all wells into one 15 mL conical tube. If passaging a single well, omit this step.
9. Pass the organoid fragments through a 70 µm cell strainer and collect the flow-through in a 50 mL conical tube. Discard the strainer.
10. Vortex the tube containing organoid fragments gently at medium speed for 5 seconds. Immediately transfer 3 x 10 µL of fragment suspension into an empty well of a 6-well plate to create 3 separate droplets. Place the remaining fragment suspension on ice.
11. Determine the average number of organoid fragments in suspension as follows:
    a. Using a light microscope, count the number of pancreatic organoid fragments in each 10 µL droplet and determine the average number of fragments/10 µL. Only count fragments that are 30 - 100 µm.

        NOTE: If the fragment density is too high to count, dilute the suspension using Advanced DMEM/F-12 and repeat step 10.
    b. Calculate the volume required to transfer 200 fragments/well to the next passage. For each new Matrigel® dome to be seeded, add the calculated volume to a 15 mL conical tube containing 1 mL of Advanced DMEM/F-12.

    *Example:*

    3 x 10 µL fragment counts = *35, 40, 42 fragments*
    Average fragment count per 10 µL = *39 fragments*
    Volume from fragment suspension to transfer 200 fragments = *51 µL*
12. Centrifuge tubes containing 200 fragments each (prepared in step 11b) at 290 x *g* for 5 minutes. Aspirate as much of the supernatant as possible without disturbing the pellets, leaving ~5 - 10 µL in the tube (the pellet is often not visible). Place tubes on ice.
13. For subsequent steps in the passaging protocol, refer to section A steps 20 - 28.

    NOTE: Pancreatic organoids seeded with 200 fragments typically require passaging every 3 - 6 days; after the initial passage, use a 1:10 to 1:30 split ratio in subsequent passages (fragment counting is not required).

## Related Products

For related products, including specialized culture and storage media, dissociation reagents, antibodies, cytokines, and small molecules, visit www.stemcell.com, or contact us at techsupport@stemcell.com.