

# TECHNICAL BULLETIN

## Mouse Pancreatic Exocrine Organoid Culture: Supplementary Protocols

The following are supplementary protocols for the culture of pancreatic exocrine organoids with PancreaCult™ Organoid Growth Medium (Mouse) (Catalog #06040). For complete culturing methods, use this document in coordination with the Product Information Sheet (PIS; Document #DX22146), which includes a full materials list and step-by-step instructions for isolating tissue fragments from a mouse pancreas, culturing pancreatic exocrine organoids in Corning® Matrigel® domes and passaging cultures as organoid fragments.

This table summarizes the available protocols for culturing pancreatic exocrine organoids in PancreaCult™ Organoid Growth Medium (Mouse) and where the detailed protocols can be located. Within this table, “Tech Bul.” refers to this document (DX27088) and “PIS” refers to the Product Information Sheet (DX22146).

	CULTURE IN MATRIGEL® DOMES		CULTURE IN A DILUTE MATRIGEL® SUSPENSION	
	As tissue or organoid fragments	As single cells	As tissue or organoid fragments	As single cells
Primary culture from fresh pancreatic tissue	PIS Section A	Tech Bul. Section 2.1	Tech Bul. Section 3.1	Tech Bul. Section 2.1
Passaging of established organoids	PIS Section B	Tech Bul. Section 2.1	Tech Bul. Section 3.2	Tech Bul. Section 3.2

## Table of Contents

- 1 General Recommendations for Pancreatic Organoid Culture
  - 1.1 Pre-wetting Plasticware
  - 1.2 Recommendations for Seeding Density
  - 1.3 When to Passage Pancreatic Exocrine Organoids
  - 1.4 Mechanical Trituration and Enumeration of Organoid Fragments
- 2 Dissociation of Pancreatic Tissue and Organoids into Single Cells
  - 2.1 Dissociating Primary Pancreatic Tissue into Single Cells
  - 2.2 Dissociating Pancreatic Exocrine Organoids Cultured in Matrigel® Domes into Single Cells
- 3 Initiation and Passaging of Pancreatic Exocrine Organoid Cultures in a Dilute Matrigel® Suspension
  - 3.1 Initiating Pancreatic Exocrine Organoid Cultures in a Dilute Matrigel® Suspension
  - 3.2 Passaging Pancreatic Exocrine Organoids in a Dilute Matrigel® Suspension
- 4 Cryopreservation of Mouse Pancreatic Exocrine Organoids

# 1 General Recommendations for Pancreatic Organoid Culture

## 1.1 Pre-wetting Plasticware

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.

To pre-wet 15 mL conical tubes, add 5 mL of Anti-Adherence Rinsing Solution (Catalog #07010) and swirl to thoroughly coat the tube. Transfer the Anti-Adherence Rinsing Solution to the next tube and repeat until all required tubes have been coated. Repeat these rinsing steps with the same volume of Advanced DMEM/F-12 (Thermo Fisher #12634010). To pre-wet 50 mL conical tubes, use the same procedure with 30 mL of each solution. Tubes may be capped and stored at room temperature (15 - 25°C) until use. To pre-wet serological pipettes and pipette tips, add 10 mL of Anti-Adherence Rinsing Solution to one 15 mL conical tube and 10 mL of Advanced DMEM/F-12 to a second 15 mL tube. Rinse the pipette or pipette tip with the two solutions in sequence immediately before use.

## 1.2 Recommendations for Seeding Density

For best results it is recommended to seed pancreatic tissue fragments harvested from one mouse pancreas (as per the PIS) into 12 wells (one 30  $\mu$ L Matrigel® dome per well), or into 5 wells of 5,000 cells/well harvested from ¼ of a mouse pancreas if seeding cultures as single cells, as per Section 2.1 (below). Once organoids are established (p1+), seed organoid cultures with 200 organoid fragments (PIS Section B) or 5,000 - 10,000 single cells per 30  $\mu$ L Matrigel® dome (Section 2.2 below).

It is crucial not to over seed the organoid cultures. If too much starting material is embedded in a Matrigel® dome, organoids cannot expand properly and the structural stability of the matrix can be compromised.

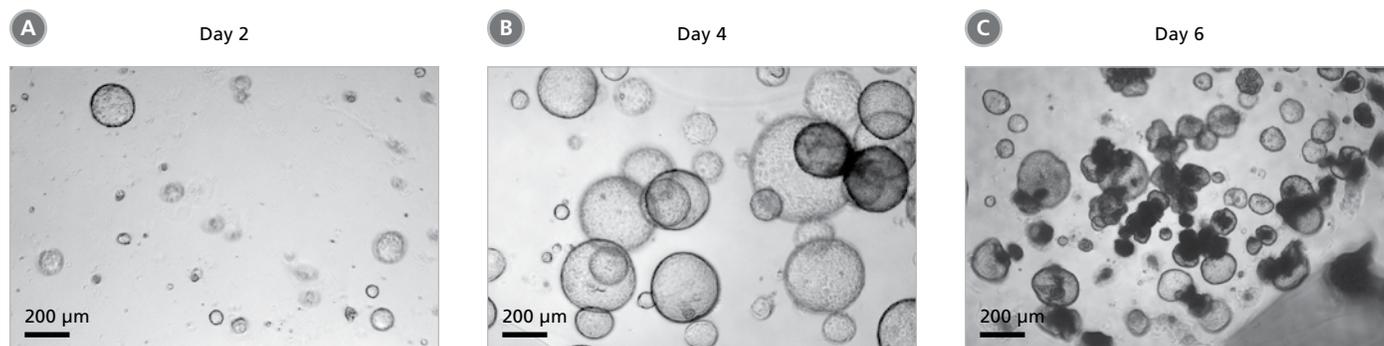
## 1.3 When to Passage Pancreatic Exocrine Organoids

Organoid cultures are typically ready to passage every 4 - 7 days during primary culture (p0) or 3 - 5 days after passaging (p1+). Passage organoids as soon as the lumens of the organoids begin to darken as the optimal time to passage may vary between cultures and is dependant on several factors including seeding density and the originating animal. If the majority of organoids are smaller than 100  $\mu$ m and exhibit clear lumens, return the culture to the incubator for an additional day of culture.

If the majority of organoids contain darkened lumens or are collapsed (Figures 1C and 2C), the culture may still be recovered through passaging. During fragment enumeration, be careful to only count organoid fragments and not single cells (Figure 3) and seed the passaged culture with 200 fragments per well. Darkened and collapsed cultures may also be recovered through single-cell passaging (Section 2.2 below) as it enables selective expansion of the healthy stem cell population.

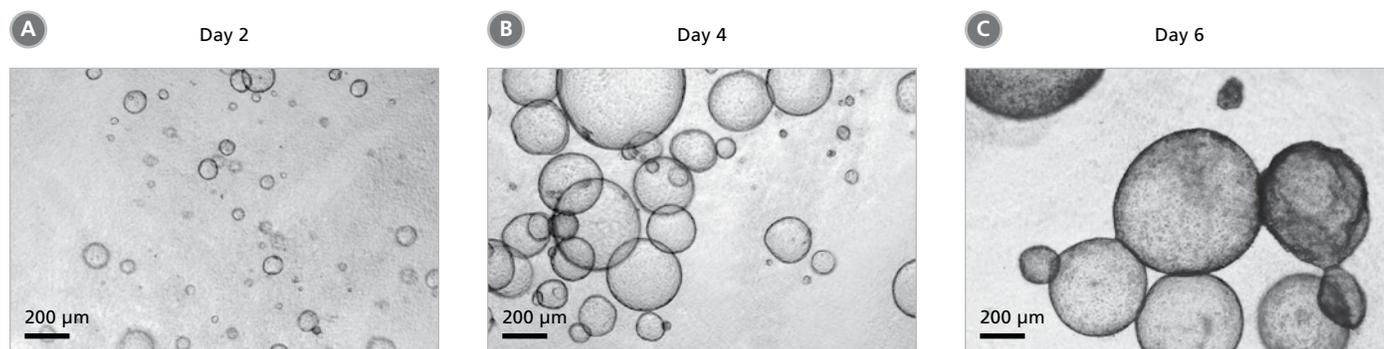
## 1.4 Mechanical Trituration and Enumeration of Organoid Fragments

Expanded organoids are mechanically triturated during passaging and the resulting organoid fragments should be smaller than 100  $\mu$ m (Figure 3A). If the majority of fragments are larger than 100  $\mu$ m (Figure 3B), continue trituration to further break up fragments before organoid fragment enumeration. When counting organoid fragments for plating, count only those that are smaller than 100  $\mu$ m and be careful not to count single cells (Figure 3C).



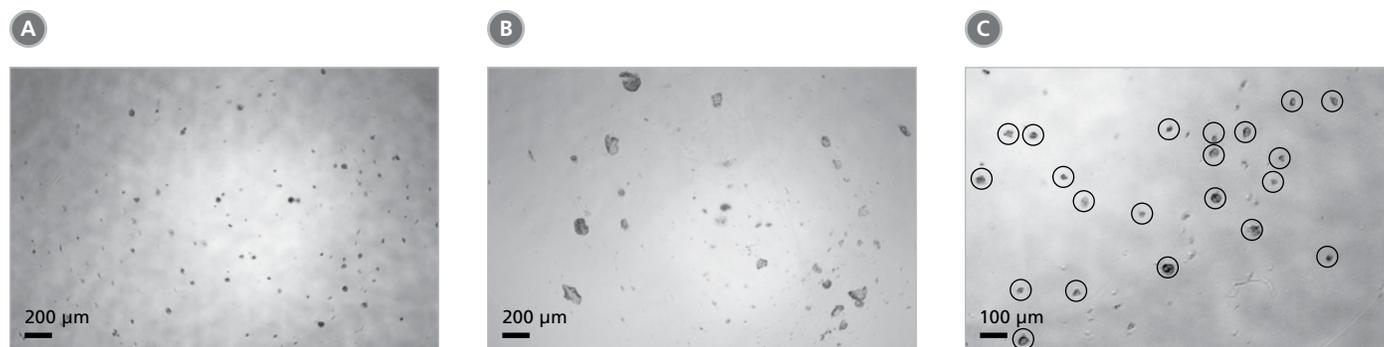
**Figure 1.** Pancreatic Organoids Grown in PancreaCult™ Organoid Growth Medium (Mouse) Plated in Matrigel® Domes.

(A) The majority of pancreatic organoids are still less than 100 µm in diameter, exhibit clear lumens and are not yet ready for passaging. (B) The majority of organoids are larger than 100 µm and the lumens of a few organoids have turned dark. These cultures are ready to be passaged. (C) Organoids have passed the typical passage window; the lumens of most organoids have darkened and many organoids have collapsed.



**Figure 2.** Pancreatic Organoids Grown in PancreaCult™ Organoid Growth Medium (Mouse) Plated in a Dilute Matrigel® Suspension.

(A) The majority of pancreatic organoids are still less than 100 µm in diameter, exhibit clear lumens and are not yet ready for passaging. (B) The majority of organoids are larger than 100 µm and the lumens of a few organoids have turned dark. These cultures are ready to be passaged. (C) Organoids have passed the typical passage window; the lumens of most organoids have darkened and some organoids have collapsed.



**Figure 3.** Pancreatic Organoids are Broken Down to 100 µm fragments for passaging.

(A) Mechanical disruption of organoids during passaging should produce organoid fragments between 30 - 100 µm in diameter. (B) Organoid fragments larger than 100 µm should be further triturated until they are 30 - 100 µm in diameter. (C) During enumeration, count fragments (circled) and ignore single cells.

## 2 Dissociation of Pancreatic Tissue and Organoids into Single Cells

### 2.1 Dissociating Primary Pancreatic Tissue into Single Cells

1. Prepare complete PancreaCult™ Organoid Growth Medium (Mouse); refer to the PIS. When initiating or passaging pancreatic exocrine organoids as single cells, supplement complete PancreaCult™ Organoid Growth Medium (Mouse) with 10  $\mu$ M Y-27632 (Catalog #72302).
2. Prepare the DNase I + TrypLE™ solution by adding 50  $\mu$ L of 1 mg/mL DNase I Solution (Catalog #07469) to 4 mL of TrypLE™ Express Enzyme (Thermo Fisher #12605010). Mix thoroughly and store on ice.
3. Process a single mouse pancreas to obtain appropriate tissue fragments as described in steps A.1 - 14 of the PIS. The following instructions pertain to proceeding with  $\frac{1}{4}$  of the pancreatic material harvested and pooled in the supernatant at the end of step A.14.
4. Centrifuge the tube at 290 x *g* for 5 minutes. Aspirate as much of the supernatant as possible without disturbing the pellet, leaving ~5 - 10  $\mu$ L in the tube.
5. Add 1 mL of DNase I + TrypLE™ to the pellet (prepared in step 2.1.2). Using a 1000  $\mu$ L pre-wetted pipette tip, pipette up and down 5 times to resuspend the pellet. Add additional DNase I + TrypLE™ to a total volume of 4 mL. Place in a 37°C water bath for 10 minutes to dissociate the collected tissue fragments into single cells.
6. Using a 5 mL pre-wetted serological pipette, pipette the single-cell suspension up and down 5 times. Add 8 mL of Advanced DMEM/F-12.
7. Centrifuge at 290 x *g* for 5 minutes at 2 - 8°C. Aspirate as much of the supernatant as possible without disturbing the pellet, leaving ~5 - 10  $\mu$ L in the tube.
8. Using a 10 mL pre-wetted serological pipette, add 5 mL of Advanced DMEM/F-12 and resuspend the pellet by pipetting up and down 5 times.
9. Pass the cell suspension through a pre-wetted 37  $\mu$ m Reversible Strainer attached to a 15 mL conical tube and collect the flow-through. Rinse the tube that contained the cell suspension with 2.5 mL of Advanced DMEM/F-12 and pass the rinse through the strainer into the same tube. Discard the strainer.
10. Using a hemocytometer, perform a cell count to estimate the number of cells in suspension.
11. Add 5000 cells to each of 5 pre-wetted 15 mL conical tubes, each containing 1 mL of cold Advanced DMEM/F-12. Aliquot cells carefully as it is important not to over seed the cultures (See Section 1.2 above).
12. Centrifuge tubes at 290 x *g* for 5 minutes. Aspirate as much of the supernatant as possible without disturbing the pellets, leaving ~5 - 10  $\mu$ L in the tubes. Place tubes on ice.
13. Plate pancreatic progenitor cells according to steps A.20 - 29 of the PIS for culture in Matrigel® domes or steps 3.1.6 - 3.1.15 to culture cells in a dilute Matrigel® suspension (below).

### 2.2 Dissociating Pancreatic Exocrine Organoids Cultured in Matrigel® Domes into Single Cells

1. Process Matrigel® domes as described in steps B.1 - 9 of the PIS.
2. Centrifuge the tube at 290 x *g* for 5 minutes. Without disturbing the pellet, carefully aspirate the supernatant, leaving ~5 - 10 µL in the tube.
3. Add 500 µL of Gentle Cell Dissociation Reagent (Catalog #07174) to the pellet and pipette up and down 5 - 10 times to mix. Place in a 37°C water bath for 10 minutes to dissociate organoid fragments into single cells.
4. Add 500 µL of Advanced DMEM/F-12 to the tube.
5. Centrifuge the tube 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.
6. Add 1 mL of cold Advanced DMEM/F-12 to resuspend the pellet. Using a hemocytometer, perform a cell count to estimate the number of cells in suspension.
7. Add 5000 - 10,000 cells per well to be seeded to the corresponding number of 15 mL conical tubes, each containing 1 mL of cold Advanced DMEM/F-12. Aliquot cells carefully, as it is important not to over seed the cultures (See Section 1.2).
8. Centrifuge tube(s) at 290 x *g* for 5 minutes. Without disturbing the pellet, carefully aspirate as much of the supernatant as possible, leaving ~5 - 10 µL in the tube. Place tube(s) on ice.

*NOTE: The pellet is often not visible.*

9. For subsequent steps in the passaging protocol, refer to steps A.20 - 29 of the PIS.

### 3 Initiation and Passaging of Pancreatic Exocrine Organoid Cultures in a Dilute Matrigel® Suspension

In addition to culture in Matrigel® domes, pancreatic exocrine organoids may be cultured in a dilute Matrigel® suspension. When cultured in suspension, Matrigel® forms a semi-solid 'cloud' that loosely encapsulates the growing organoids. Growth in suspension allows the scale-up of cultures beyond what may be practical when plating in Matrigel® domes. Such scale-up is possible due to the elimination of medium changes, decreased reliance on the physical stability of the Matrigel®, and a plating technique that is more amenable to high-throughput work-flows. Compared to organoids grown in Matrigel® domes, organoids grown in a dilute Matrigel® suspension are typically ready for passage earlier, usually within 3 - 6 days and can be maintained in culture through passaging or cryopreserved for future experiments.

#### 3.1 Initiating Pancreatic Exocrine Organoid Cultures in a Dilute Matrigel® Suspension

1. Place a wrapped 12-well plate for suspension culture (Greiner Bio-One #665102) at 2 - 8°C for at least 10 minutes. Place boxes of sterile 1000 µL and 200 µL pipette tips at 2 - 8°C.
2. Thaw 500 - 1000 µL of Matrigel® on ice.
3. Prepare complete PancreaCult™ Organoid Growth Medium (Mouse) and place on ice. For complete instructions refer to the PIS.
4. Prepare Tissue Dissociation Cocktail (see PIS). Warm to room temperature (15 - 25°C).
5. Process mouse pancreatic tissue to harvest duct fragments as described in steps A.4 - 19 in the PIS or follow section 2.1 *Dissociating Primary Pancreatic Tissue into Single Cells* (above).
6. Using a cooled pipette tip, add 100 µL of thawed Matrigel® to 900 µL of cold PancreaCult™ Organoid Growth Medium (Mouse) in a 15 mL conical tube for each well to be plated (example if plating 10 wells, the tube should contain 1 mL of Matrigel® combined with 9 mL of cold PancreaCult™ Organoid Growth Medium (Mouse)). Mix thoroughly and store on ice.
7. Place the cooled 12-well plate on ice and add 950 µL of the Matrigel®/PancreaCult™ mixture (prepared in step 3.1.6) to each well to be plated.
8. Process one tube/pellet from step A.19 at a time, as described below. Work quickly after adding Matrigel® to the pellet to ensure the Matrigel® does not solidify.
9. Using a pipettor with a cooled 200 µL pipette tip, add 50 µL of the Matrigel®/PancreaCult™ mixture (prepared in step 3.1.6) on top of the pellet.
10. Gently mix the suspension by pipetting up and down 5 - 8 times. Pipette only to the first stop to avoid introducing air bubbles.
11. Pipette the entire suspension into 1 well of the 12-well plate containing 950 µL of the Matrigel®/PancreaCult™ mixture (prepared in step 3.1.7). Gently agitate plate to mix contents of the well.
12. Repeat steps 3.1.9 - 11 for the remaining tubes, adding the 50 µL Matrigel®/PancreaCult™ cell suspension into a fresh well already containing 950 µL of the Matrigel®/PancreaCult™ mixture.
13. Place the lid on the culture plate. Place plate on an orbital shaker set to 80 rpm in a 37°C incubator with 5% CO<sub>2</sub>.
14. At 10 minutes after seeding the wells, capture one 2X image per well using a brightfield microscope (Day 0 images). Carefully return plate to incubator.  
*NOTE: Once cultures have been placed on an orbital shaker, the Matrigel® added to each well will often form a semi-solid 'cloud' in which fragments are encapsulated.*
15. Take photos of the wells every other day, or at desired time points, to track the growth of organoids until they are passaged. No medium changes are necessary.

## 3.2 Passaging Pancreatic Exocrine Organoids in a Dilute Matrigel® Suspension

1. Place a wrapped 12-well plate for suspension culture at 2 - 8°C for at least 10 minutes (Greiner Bio-One #665102). Place boxes of sterile 1000 µL and 200 µL pipette tips at 2 - 8°C.
2. Thaw Matrigel® on ice (~110 µL/well to be passaged).
3. Prepare complete PancreaCult™ Organoid Growth Medium (Mouse) (see PIS). Place on ice.
4. Using a 1000 µL pipettor, determine the volume of the contents of each well. Set the pipettor to this volume, then vigorously pipette the mixture up and down 30 times, taking care to minimize the generation of bubbles.

*NOTE: This results in mechanical breakdown of pancreatic 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 (see Section 1.4).*

5. If passaging multiple wells, combine the contents of all wells into a single 15 mL conical tube. If passaging a single well, transfer the entire volume of the well into one 15 mL conical tube.
6. To prepare organoid fragments for plating, refer to steps B.9 - 12 of the PIS. To prepare single cells for plating, refer to steps 2.2.2 - 2.2.8.
7. For subsequent steps in the passaging protocol, refer to steps 3.1.6 - 3.1.15.

*NOTE: Monitor pancreatic exocrine organoids daily. 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.*

# Mouse Pancreatic Exocrine Organoid Culture: Supplementary Protocols

## 4 Cryopreservation of Mouse Pancreatic Exocrine Organoids

1. Place Advanced DMEM/F-12 and CryoStor® CS10 (Catalog #07930) in a biosafety cabinet on ice.
2. Place labeled 1.8 mL cryogenic vials in tube racks until ready to use. Immediately before use, place vials in the biosafety cabinet on ice.
3. To prepare organoid fragments for cryopreservation, refer to steps B.4 - 11 of the PIS. Using the method outlined in step B.11 calculate the total volume required to transfer 800 organoid fragments per vial to be frozen (volume for 800 organoid fragments x # of vials). Add this total volume to a single 15 mL conical tube containing 1 mL of Advanced DMEM/F-12.
4. Centrifuge tube 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.
5. Uncap 1.8 mL cryogenic vials on ice to minimize delays in subsequent steps.
6. Gently resuspend pellet 5 - 8 times in 1 mL cold CryoStor® CS10.
7. Add an additional 1 mL of cold CryoStor® CS10 for every vial to be cryopreserved and gently mix the suspension an additional 5 - 8 times by pipetting.
8. Using a 1000 µL pipette, aliquot 1 mL of this CryoStor®/fragment suspension into labeled and cooled 1.8 mL cryogenic vials, mixing the total volume in the conical tube 1 - 2 times prior to dispensing into each tube to ensure even distribution of fragments.
9. Immediately cap and transfer all cryogenic vials containing fragments into a controlled-rate cell freezing container. Place cell freezing container at -80°C.
10. Cryogenic vials should be transferred to liquid nitrogen storage after 24 - 48 hours of storage at -80°C.

*NOTE: For a complete thawing protocol, refer to the Mouse Pancreatic Organoids PIS (Document #DX22148).*

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