ESTABLISH & MAINTAIN MOUSE PANCREATIC ORGANOIDS

PancreaCult™ Organoid Growth Medium (Mouse)

The pancreas is a digestive and endocrine organ with roles in the break-down of starches, proteins and fats, as well as in glucose homeostasis. Despite the prevalence and importance of research into pancreatic cancer throughout the previous decades, a method for the long-term, in vitro maintenance of pancreatic exocrine tissue has only been recently described.[1,2] In this system, isolated pancreatic ducts form pancreatic exocrine organoids when grown with the appropriate growth factors and extracellular matrix.

Organoids are self-assembling, three-dimensional cell cultures that incorporate some of the cell types and key features of the represented organ of origin. Maintenance of a proliferating stem and progenitor cell population allows epithelial organoids to be maintained in culture far beyond what is possible with other forms of ex vivo primary cell culture. Due to their efficient growth in vitro and direct relevance to the pancreatic epithelium, pancreatic exocrine organoids can complement or replace many experimental methodologies for studying the pancreas.

The ability to generate organoid cultures from cells from a variety of healthy and disease-specific backgrounds provides experimental advantages compared with other common model systems. Isolation of primary tissue from mouse primary tumors and metastases allows the growth of tumor-derived organoids providing a model for pancreatic carcinomas and pancreatic ductal adenocarcinoma progression.[3] These organoids retain key characteristics of the parent tumor, including genotype and phenotype, providing a convenient model system for in vitro experimentation.

PancreaCult™ Organoid Growth Medium (Mouse) enables the growth of pancreatic exocrine organoids from pancreatic ducts, duct fragments, single cells or organoid fragments. Pancreatic organoids can be maintained through extended passaging or cryopreserved, providing a readily available source of cells for future experiments. Organoids are typically observable within 2 days of primary culture and are ready for passage after approximately 3 - 6 days. Pancreatic exocrine organoids grown in PancreaCult™ Organoid Growth Medium (Mouse) exhibit markers of pancreatic progenitors (eg. Pdx1, Axin2) and ductal cells (eg. Krt19, Muc1).

Why use PancreaCult™?

CONVENIENT. In vitro system for generating organoids within a week.

STEP-BY-STEP PROTOCOL. No injury models, hand-picking of ducts or cell sorting required.

SIMPLE, TWO-COMPONENT FORMAT. Serum-free and defined medium formulation.

FLEXIBLE PROTOCOL. Organoids can be grown from duct fragments or single cells and cultured in matrix domes or suspension.

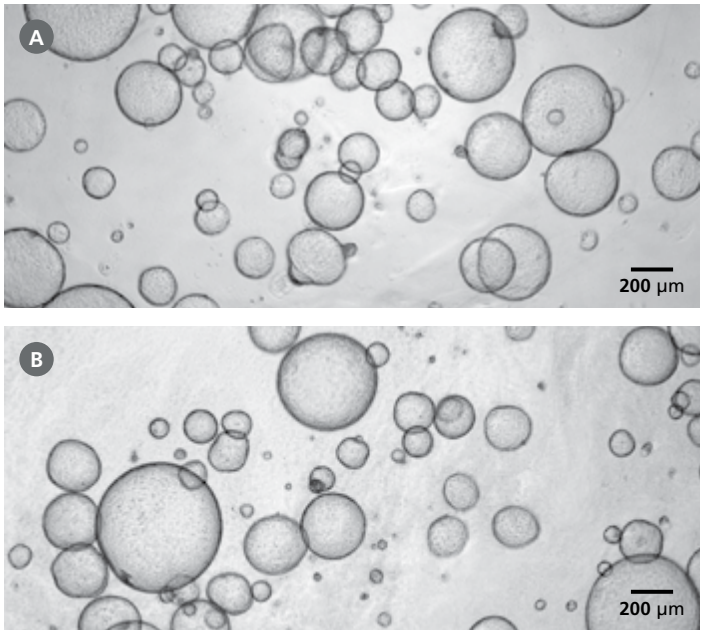

Figure 1. Organoids Grown in PancreaCult™ Organoid Growth Medium (Mouse)

Pancreatic exocrine organoids are observed within one week when cultured in (A) Corning® Matrigel® domes or (B) a dilute Matrigel® suspension. Organoids were imaged during passage 2, on day 4.

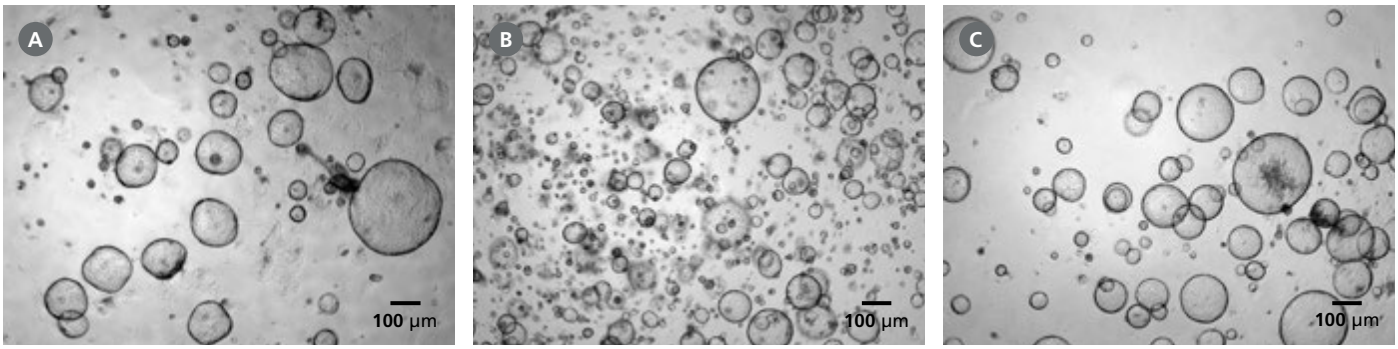

Figure 2. Mouse Pancreatic Organoids can be Initiated from a Variety of Starting Materials.

PancreaCult™ Organoid Growth Medium (Mouse) enables the initiation of pancreatic exocrine organoids from (A) duct fragments, (B) single cells and (C) cryopreserved organoid fragments. All organoids were grown in Matrigel® domes. Organoids were imaged on day 4 or day 5 of primary culture (duct fragments and single cells, respectively) or day 3 of the first passage post-thaw (cryopreserved organoids).

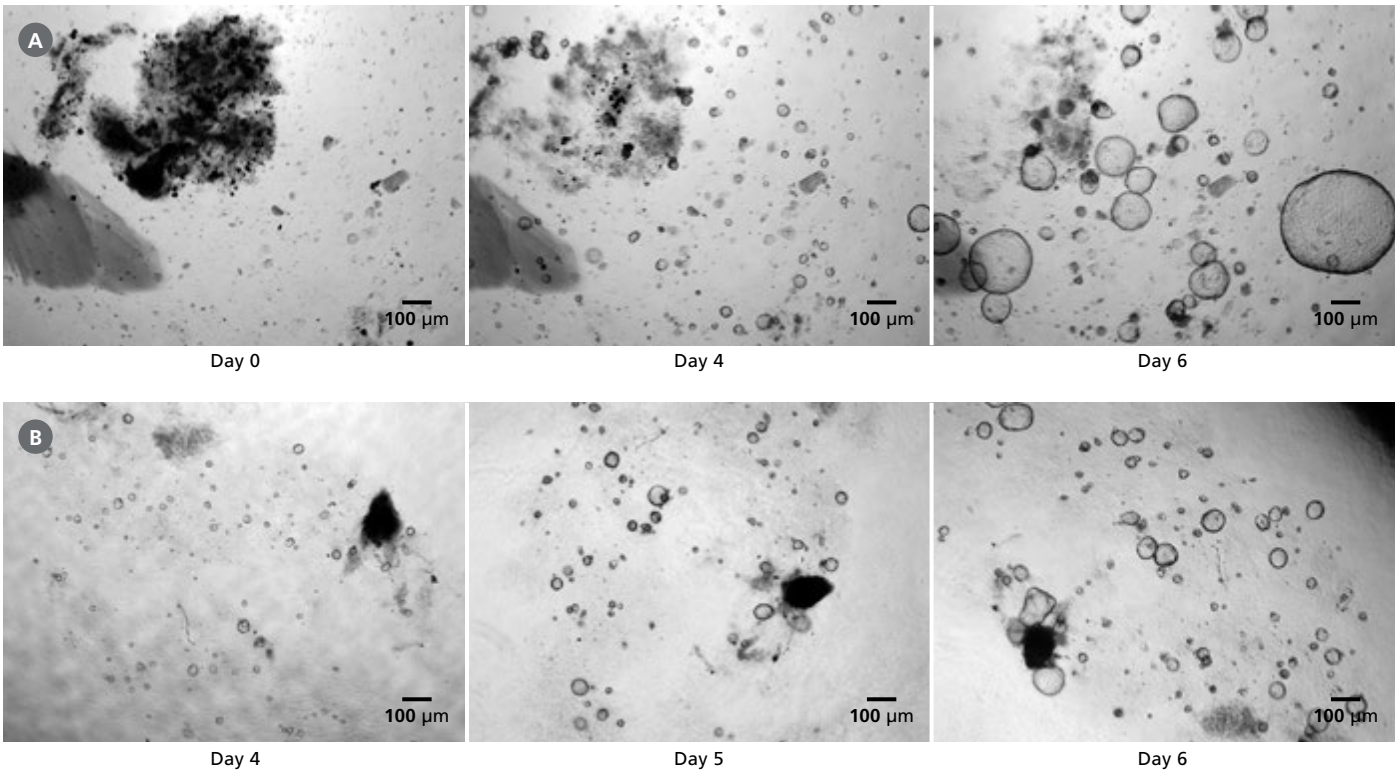

Figure 3. Pancreatic Organoids can be Grown in Matrigel® Domes or as a Dilute Matrigel® Suspension.

Organoids cultured using PancreaCult™ Organoid Growth Medium (Mouse) from freshly isolated pancreatic tissue fragments and plated in (A) Matrigel® domes or (B) as a dilute Matrigel® suspension. Organoids grown in either culture condition are typically ready for passage within 3 - 6 days.

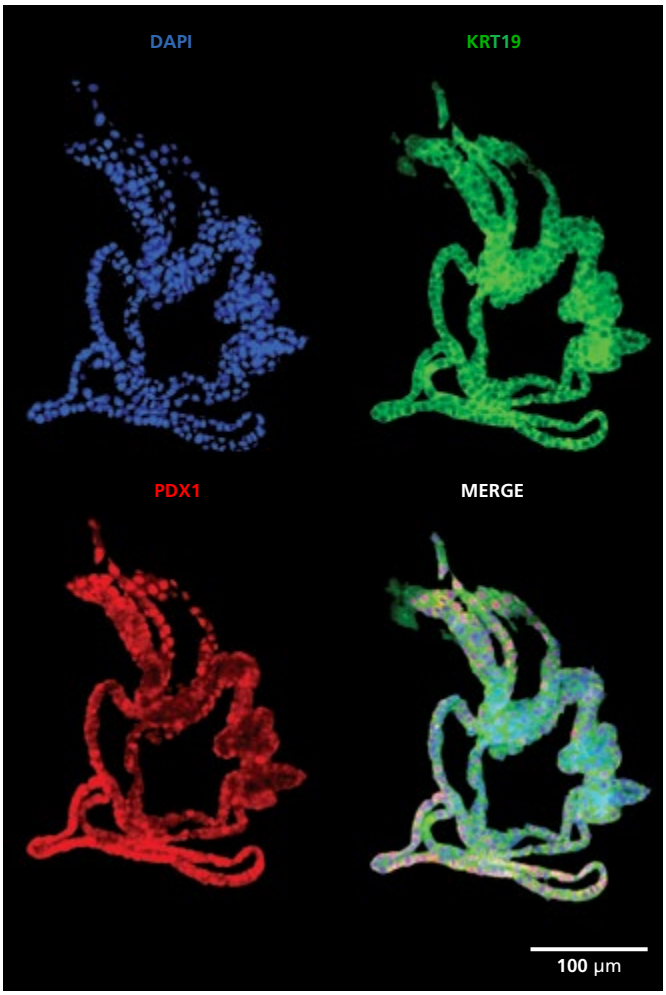

Figure 4. Pancreatic Exocrine Organoids Display Markers of Pancreatic Progenitor and Ductal Cells.

Pancreatic exocrine organoids grown in PancreaCult™ and stained for nuclei (DAPI, blue), ductal marker KRT19 (green) and pancreatic progenitor marker PDX1 (red). Organoids were imaged during passage 12 on day 5.

Note: The folded appearance of epithelium is a function of cryosectioning and not representative of the shape of proliferating organoids.

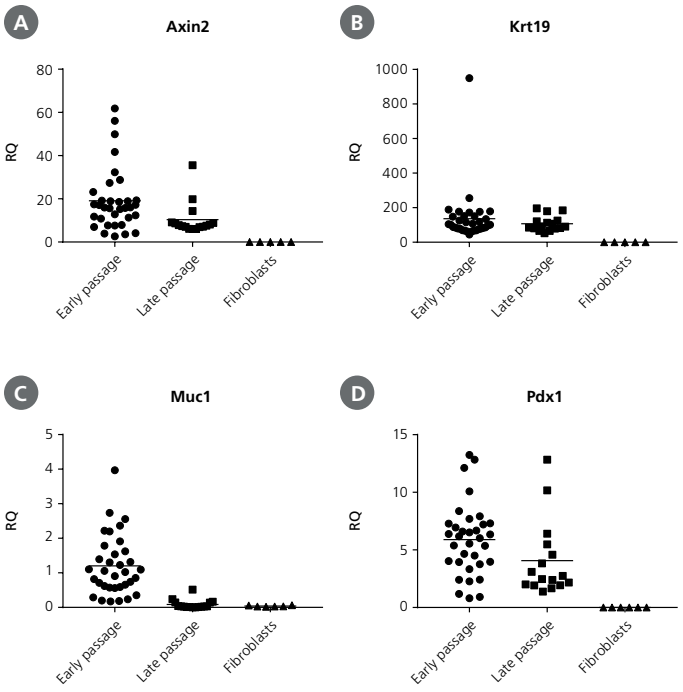

Figure 5. Pancreatic Exocrine Organoids Retain Pancreatic Marker Expression During Passaging.

Pancreatic organoids express stem cell markers and those typical of the pancreatic exocrine system, including (A) Axin2, (B) Krt19, (C) Muc1 and (D) Pdx1. Relative quantification (RQ) of each marker is reported relative to the 18S and TBP housekeeping genes and normalized to C57/Bl6 pancreatic tissue. Marker expression was assayed during early passages (passage 1-5) and late passages (passage 6-10).

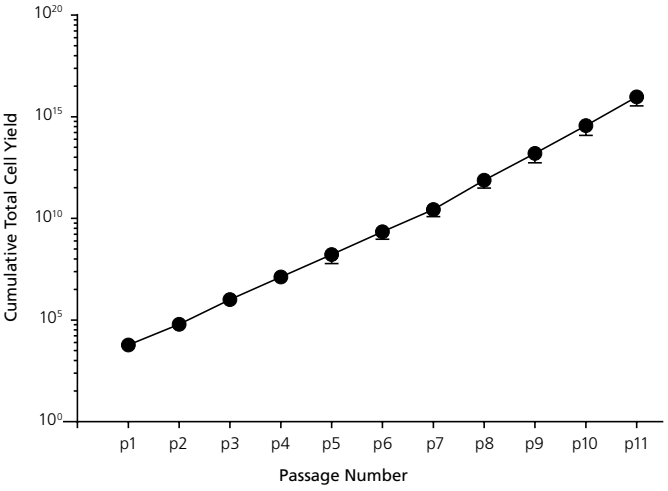

Figure 6. Expansion of Organoids Grown in PancreaCult™ Organoid Growth Medium (Mouse).

Organoids cultured with PancreaCult™ Organoid Growth Medium (Mouse) show efficient growth over multiple passages. Cultures were split with an average split ratio of 1:16 at each passage.

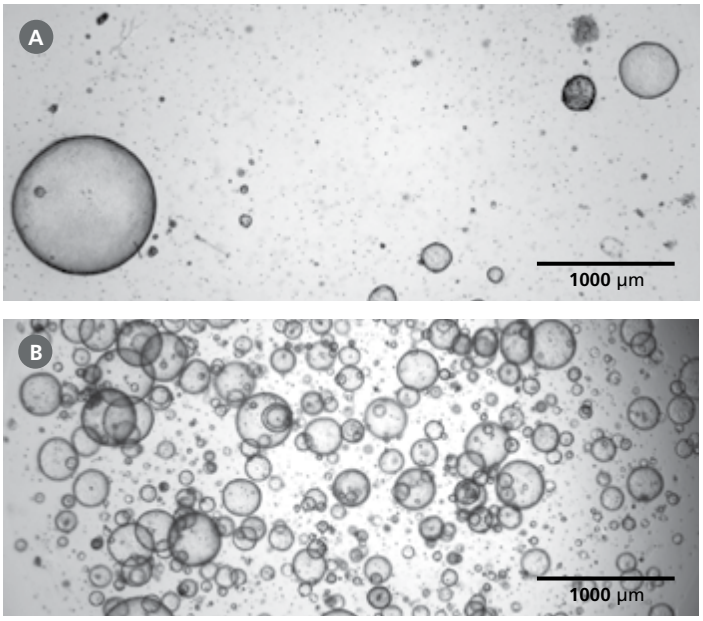

Figure 7. Pancreatic Exocrine Organoids Provide a Model for Pancreatic Carcinomas.

PancreaCult™ Organoid Growth Medium (Mouse) supports the growth of organoids from pancreatic carcinomas. Pancreatic ducts were isolated from KPC mice ($Kras^{+/LSL\text{-}G12D}$; $Trp53^{+/LSL\text{-}R172H}$; Pdx1-Cre) and cultured in PancreaCult™ Organoid Growth Medium (Mouse). Organoids were imaged on (A) day 4 of primary culture and (B) day three after the first passage. An activated KRAS genotype was retained in organoids during culture. Data used with permission from Dr. David Tuveson.

Product Information

PRODUCT	CATALOG #
PancreaCult™ Organoid Growth Medium (Mouse)	06040
Mouse Pancreatic Organoids	70933
Anti-Adherence Rinsing Solution	07010
Collagenase IV	07909
Dispase	07923
CryoStor® CS10	07930
37 µm Reversible Strainer	27250

WALLCHART

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VIDEO

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References

1. Huch M, et al. (2013) Unlimited in vitro expansion of adult bi-potent pancreas progenitors through the Lgr5/R-spondin axis. EMBO J. 32(20): 2708-21.
2. Broutier L., et al. (2016) Culture and establishment of self-renewing human and mouse adult liver and pancreas 3D organoids and their genetic manipulation. Nature Protocols. 11(9): 1724-43.
3. Boj SF, et al. (2015) Organoid Models of human and mouse ductal pancreatic cancer. Cell. 160(1-2): 324-38.