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# Mouse Pancreas Dissection and Fixation for Cryosectioning

Islet and Pancreas Analysis Core [1]

[1]Vanderbilt Diabetes Research Center



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This SOP defines the method used by the Vanderbilt Diabetes Center Islet and Pancreas Analysis (IPA) Core for fixation and embedding of mouse pancreas.

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Reagents:

- Sodium phosphate, dibasic ($Na_2HPO_4$), Sigma #S7907
- Sodium chloride (NaCl), Sigma #S7653
- Potassium chloride (KCl), Fisher Scientific #BP366
- 16% Paraformaldehyde (PFA), Electron Microscopy Sciences #15710
- Sucrose, Fisher Scientific #BP220-1
- Ketamine, Henry Schein #9952949
- Xylazine, Henry Schein #4015809
- 10mM PBS, Gibco #14190144
- Optimum cutting temperature (OCT) compound, Fisher Scientific #50-363-579

Supplies and tools:

- 100 mm tissue culture dishes, Fisher Scientific #08-772-32 or similar
- 15 mL conical tubes, Corning #430828 or similar
- Cryomolds, VWR #25608-916 or similar
- Superfrost Plus Gold slides, Fisher Scientific #1518848
- Forceps, Roboz # RS-8254 or similar
- Aluminum foil
- Zip-seal plastic bags
- Dry ice (pellet or block)

Equipment:

- Platform rocker, Corning LSE #07-202-200 or similar

## Reagent preparation

1 **Ketamine (15 mg/mL)/xylazine (3 mg/mL) working solution** – Use 5 mL syringes with 18G needles. Label all bottles with additive, mix date, and expiration date of earliest-expiring ingredient.

1.1 Pull 2 mL of xylazine and inject into a 10 mL bottle of ketamine.

1.2 Pull 2 mL of ketamine/xylazine and inject into a 10 mL bottle of saline.

2 **0.1 M PBS** (1L) – Dissolve 12.07 g $Na_2HPO_4$ (dibasic), 8.0 g NaCl, and 2.0 g KCl in 1L Milli-Q water. This solution should be made fresh or can be sterile filtered, kept at **4 °C** , and used



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within 1 month.

3 **4% Paraformaldehyde (PFA)** – Dilute one ampule (10 mL) 16% PFA in 30 mL 0.1M PBS. This solution should be made fresh the day of fixation. Approximately 13mL per pancreas is required.

3.1 Pipet 13 mL aliquots into labeled 15 mL conical tubes (one per pancreas). Using analytical balance, record weight of each tube to nearest 0.01 g.

4 **30% Sucrose** – Dissolve 15 g sucrose in 35mL 10 mM PBS. Solution can be placed on shaker to ensure sucrose fully dissolves. Approximately 13 mL per pancreas is required.

## Pancreas dissection and fixation

5 Inject mice intraperitoneally with **ketamine/xylazine solution** (1 µl/mg body weight) using a 1 mL syringe with a 27G needle. Place mice into a plastic bin with air holes.

5.1 After 5-10 minutes, check to see if mice are fully anesthetized bygently pinching feet and tail. If there is a jerking or squeaking response, inject about 100 µL more of ketamine/xylazine solution, and wait a few more minutes. Continue checking for full anesthetization before beginning surgery.

6 Place mouse face up on the surgical stage, tape limbs down, and wipe with 70% ethanol. Open the body cavity using forceps and scissors and locate pancreas by moving stomach and intestines to the side. Use two pairs of forceps to separate the pancreas from the duodenum and stomach, starting from the head. Remove the pancreas with the spleen still attached.



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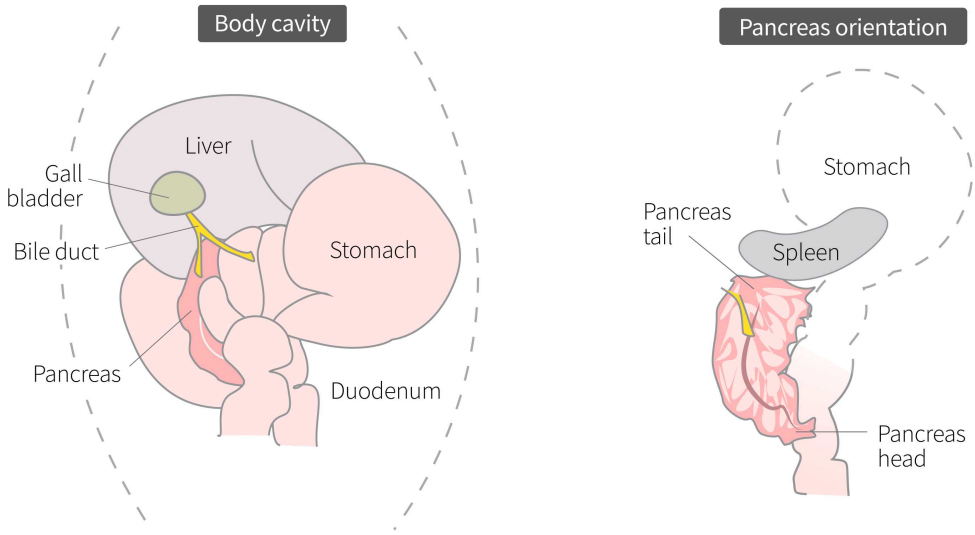


**Above left:** Pancreas location relative to other internal organs.
**Above right:** Pancreas tail attaches to spleen; pancreas head attaches to duodenum.

7 Place the pancreas in a 100 mm tissue culture dish containing 10 mL ice-cold **10 mM PBS**. Dissect away the spleen and remove all fat tissue attached to pancreas.

If there is excess bleeding during dissection, rinse pancreas in an additional 10 mL ice-cold 10 mM PBS before fat removal.

8 Place pancreas into preweighed 15 mL tube with **4% PFA** (step 3.1). Using analytical balance, record weight of tube containing pancreas to nearest 0.01 g.

9 Fix pancreas for **02:00:00** on ice with gentle agitation using a shaker at low speed.

10 Decant fixative and wash pancreas 4 times in approximately 13 mL **0.1 M PBS** for **00:30:00** . Tubes should be kept on ice for washes with gentle agitation using a shaker at low speed.

11 Decant PBS and add approximately 13 mL **30% sucrose**. Store **Overnight** at **4 °C** to



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cryoprotect pancreas.

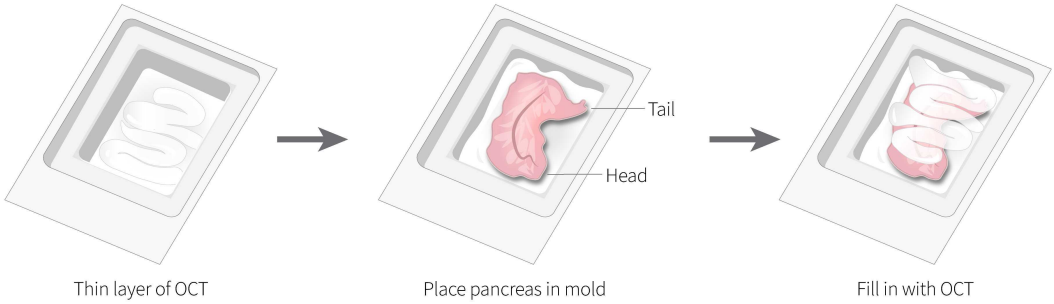
## Embedding and cryosectioning

12 Coat a labeled cryomold with a thin layer of OCT compound and set aside.

13 Retrieve 15 mL conical tube with pancreas in 30% sucrose (step 11) and pour contents into a 100 mm tissue culture dish.

13.1 Using fine forceps, pick up pancreas and blot excess sucrose from tissue using Kimwipes.

14 Place the pancreas into the cryomold as shown below, using forceps to gently push the tissue to the bottom.




**Orientation of pancreas in cryomold.**

15 Place cryomold with pancreas onto a layer of dry ice. Fill remaining volume of cryomold with OCT, covering the pancreas, and let freeze thoroughly on dry ice until OCT is opaque.

16 Wrap frozen cryomold in pre-labeled square of aluminum foil, place into a sealed plastic bag, and store at **-80 °C** until ready to cryosection.

17 Cut cryosections at 5-10 µm thickness and mount to Plus Gold charged slides for staining. For mass quantification, collect cryosections throughout the entire pancreas and to obtain at least 5 sections 200 µm apart.



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Immunofluorescent Staining of Mouse Pancreas for Islet Cell Mass Analysis

**by Islet and Pancreas Analysis Core, Vanderbilt Diabetes Research Center**

PREVIEW RUN



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