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🌐 Mouse Pancreatic Islet Isolation

Nancy Smith¹, Aliya F Spigelman¹, Haopeng Lin¹,
Patrick E Macdonald¹

¹University of Alberta

CIRTNR2FIC

Aliya F Spigelman
University of Alberta

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Protocol status: Working
We use this protocol and it's working

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ABSTRACT

This protocol details islet isolation from mouse pancreas. The protocol is divided into 3 main parts; *in situ* perfusion of pancreas with collagenase, pancreas digestion, and islet purification. A link is provided for the purification using histopque gradient.

GUIDELINES

Digestion time using collagenase from *Clostridium histolyticum* type V is lot specific. Lot #010M8620 has a 7 minutes digesting time. New lots require testing and digestion time must be adjusted for the new lot's activity.

Keywords: islets, isolation, pancreas, mouse, perfusion, purification, collagenase

MATERIALS

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- ✘ FBS (Canadian Origin) **Gibco - Thermo Fischer Catalog #12483-020**
- ✘ Sodium bicarbonate **Sigma Aldrich Catalog #S5761**
- ✘ 5cc syringes **BD Biosciences Catalog #302832**
- ✘ Extra fine Iris scissors **Fine Science Tools Catalog #14084-08**
- ✘ Adson serrated forceps **Fine Science Tools Catalog #11006-12**
- ✘ Adson 1x2 teeth forceps **Fine Science Tools Catalog #10027-12**
- ✘ Curved Forceps **Fine Science Tools Catalog #11001-12**
- ✘ Halsted-Mosquito hemostats **Fine Science Tools Catalog #13009-12**
- ✘ Ultra Fine point forceps **Fine Science Tools Catalog #11370-40**
- ✘ Gibco Penicillin-Streptomycin (10000 U/ml) **Fisher Scientific Catalog #15-14122**
- ✘ 30G needles **BD Biosciences Catalog #B305106**
- ✘ Gibco RPMI 1640 **Thermo Fisher Scientific Catalog #11875**
- ✘ Hanks Balanced Salts (HBSS) **Sigma Aldrich Catalog #H6136**
- ✘ Collagenase from Clostridium histolytic type V lot #010M8620 **Sigma Aldrich Catalog #C9263**

BEFORE START INSTRUCTIONS

Hanks' Balanced Salts (HBSS) and Mouse Islet Culture Media should be prepared in advance.

Solution Prep- Hanks' Balanced Salts (HBSS) - Sigma H6136

- 1 Measure out 900ml of room temperature H₂O.
- 2 While gently stirring the water, add the powdered medium. Stir until dissolved. DO NOT HEAT.
- 3 Rinse original vial with water to remove traces and add to above.

4 Add 0.35g sodium bicarbonate and stir until dissolved.

🧪 0.35 g Sodium Bicarbonate

5 Adjust pH to 7.4

6 Bring solution to 1L.

7 Store at 4°C

🌡️ 4 °C

Solution Prep - Mouse Islet Culture Media

8 Add FBS and Pen/Strep to bottle of RPMI

500ml RPMI 1640 (11.1mM glucose)	Gibco 11875 -119
50ml FBS Canadian Origin	Gibco 12483 -020
5ml Pen/strep (10000 Unit/ml/10000 ug/ml)	Gibco 15140 -122

9 Store at 4°C

🌡️ 4 °C

Solution Prep- Collagenase type V - Sigma C9263 lot 010M8..

10 Dissolve 1mg/ml Collagenase in HBSS from above.

- Approximately 5ml per mouse for injection and shaking
- Make fresh before isolation, and keep on ice (use within the hour)

Pancreas Perfusion

- 11 Euthanize mouse according to your institute's research ethics protocols. (We use a CO₂ chamber)
- 12 Make a midline incision from the lower abdomen to the sternum.
- 13 Common bile duct is tied or clamped where it meets the intestine.
- 14 Collagenase is injected *in situ* via the common bile duct using a size 30G needle until the pancreas is inflated (typically 1-2 mL). The head and the tail of the pancreas should be inflated to maximize the number of islets isolated.
- 15 The pancreas is removed and placed in tube (15mL or 50mL depends on preference) with remaining collagenase (1-2mL) and kept on ice until the next step.

Digestion

- 16 After isolation, allow digestion to proceed for 7 minutes (lot specific -Sigma C9263 lot 010M8620) in shaking water bath at 37°C.

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37 °C
- 17 Shake digested material vigorously.

- 18** Add Mouse Islet Media to stop digestion. Tubes remain on ice until ready for purification.

Purification

- 19** Allow islets to settle (2-3 minutes) and remove excess fat. Proceed to the histopaque gradient protocol to purify the islets. If hand picking is preferred, go to the next step.
<https://www.protocols.io/view/purification-of-mouse-pancreatic-islets-using-hist-u7ueznw>
- 20** Pour islets into a non tissue culture dish and pick islets into a 35mm non tissue culture dish.
- 21** Once islets picked cleanly, culture using Mouse Islet Media in 37°C, 5% CO₂ until ready for use in experiment.