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Mouse Pancreatic Islet Isolation

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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 activiity.

Materials

MATERIALS

- FBS (Canadian Origin) **Gibco - Thermo Fisher Scientific Catalog #**12483-020
- Sodium bicarbonate **Merck MilliporeSigma (Sigma-Aldrich) Catalog #**S5761
- 5cc syringes **Becton Dickinson (BD) Catalog #**302832
- Extra fine Iris scissors **Fine Science Tools Catalog #**14084-08
- Adson serrated forceps **Fine Science Tools Catalog #**11006-12
- Adson 1×2 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 #**15-140-122
- 30G needles **Becton Dickinson (BD) Catalog #**B305106
- Gibco RPMI 1640 **Thermo Fisher Scientific Catalog #**11875
- Hanks Balanced Salts (HBSS) **Merck MilliporeSigma (Sigma-Aldrich) Catalog #**H6136
- Collagenase from Clostridium histolytic type V lot #010M8620 **Merck MilliporeSigma (Sigma-Aldrich) Catalog #**C9263

Troubleshooting

Before start

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 H_2O.

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

Soultion 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 010M8620:

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 CO_2 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.
00:07:00
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% CO_2 until ready for use in experiment.