Apr 14, 2020

Mouse Pancreatic Islet Isolation

Nancy Smith[1], Aliya Spigelman[1], Haopeng Lin[1], Patrick Macdonald[1]

[1]University of Alberta

1 Works for me dx.doi.org/10.17504/protocols.io.sqaedse

Aliya F. Spigelman
University of Alberta

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.

EXTERNAL LINK

http://www.bcell.org

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

NAME	CATALOG #	VENDOR
FBS (Canadian Origin)	12483-020	Gibco - Thermo Fischer
Sodium bicarbonate	S5761	Sigma Aldrich
5cc syringes	302832	BD Biosciences
Extra fine Iris scissors	14084-08	Fine Science Tools
Adson serrated forceps	11006-12	Fine Science Tools
Adson 1x2 teeth forceps	10027-12	Fine Science Tools
Curved Forceps	11001-12	Fine Science Tools
Halsted-Mosquito hemostats	13009-12	Fine Science Tools
Ultra Fine point forceps	11370-40	Fine Science Tools
Gibco Penicillin-Streptomycin (10000 U/ml)	15-140-122	Fisher Scientific
30G needles	B305106	BD Biosciences
Gibco RPMI 1640	11875	Thermo Fisher Scientific
Hanks Balanced Salts (HBSS)	H6136	Sigma Aldrich
Collagenase from Clostridium histolytic type V lot #010M8620	C9263	Sigma Aldrich

BEFORE STARTING

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

Citation: Nancy Smith, Aliya Spigelman, Haopeng Lin, Patrick Macdonald (04/14/2020). Mouse Pancreatic Islet Isolation. https://dx.doi.org/10.17504/protocols.io.sqaedse

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.

Citation: Nancy Smith, Aliya Spigelman, Haopeng Lin, Patrick Macdonald (04/14/2020). Mouse Pancreatic Islet Isolation. https://dx.doi.org/10.17504/protocols.io.sqaedse

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.

Citation: Nancy Smith, Aliya Spigelman, Haopeng Lin, Patrick Macdonald (04/14/2020). Mouse Pancreatic Islet Isolation.
https://dx.doi.org/10.17504/protocols.io.sqaedse