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Purification of Mouse Pancreatic Islets using Histopaque Gradient Centrifugation

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1 Works for me dx.doi.org/10.17504/protocols.io.u7ueznw

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ABSTRACT

This protocol details the purification of mouse pancreatic islets using histopaque gradient centrifugation.

EXTERNAL LINK

http://www.bcell.org

MATERIALS

NAME	CATALOG #	VENDOR
Hank Balanced Salt Solution 1X	H6136	Sigma Aldrich
Albumin Bovine Serum (30%)	160928-0262	equitech bio, inc.
Histopaque 1077	1077-1	Sigma Aldrich
RPMI 1640 (11.1mM glucose)	11875-119	Gibco - Thermo Fischer
FBS Canadian Origin	12483-020	Gibco - Thermo Fischer
Cell dissociation sieve	CD1-1KT	Sigma Aldrich
50ml Falcon tubes	352070	Corning
30ml syringe	302832	BD Biosciences
18G needle	305196	BD Biosciences
Penicillin Streptomycin	15140 122	Invitrogen - Thermo Fisher
Sodium bicarbonate	S5761	Sigma Aldrich

BEFORE STARTING

1X Hanks' Balanced Salt Solution (HBSS) + BSA, Coating and Mouse Islet Culture Media should be prepared in advance and should be cold before starting gradient steps.

Solution Prep - 1X Hanks' Balanced Salt Solution (HBSS) + BSA - Sigma H6136 and Coating

1 Measure out 900ml of room temperature H_2O

900 ml MiliQ water

2 While gently stirring the water, add the powdered medium. Stir until dissolved. DO NOT HEAT.

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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 Take 34ml of the prepared 1X HBSS and add 6ml of 30% BSA (equitech bio, inc 160928-0262) in a 50ml falcon tube. This is to be used for coating.

8 Add 4ml of 30% BSA (equitech bio, inc 160928-0262) to the prepared 1X HBSS

9 Store at 4°C
4 °C

Solution Prep - Mouse Islet Culture Media

10 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

11 Store at 4°C
4 °C

Gradient Protocol

12 Once digest of pancreas in the waterbath is complete (see Mouse Pancreatic Islet Isolation protocol, Step 16)
https://www.protocols.io/view/mouse-pancreatic-islet-isolation-sqaedse
Shake 50ml falcon tube 30 times.

13 Bring up volume to 50ml by adding 1X HBSS+BSA to falcon tube.

14 Centrifuge for 2 minutes at 339 g at 4°C
02:00:00
4 °C

15 Remove supernatant

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16 Re-suspend pellet in 20ml of 1X HBSS+BSA. Pour re-suspended pellet through #60 screen (cell dissociation sieve, Sigma CD1-1KT) that has been rinsed with coating. Using a 30ml syringe filled with 1X HBSS+BSA and 18G needle rinse screen (use about 20ml). Return islets to 50ml tube and rinse the beaker with the remaining 10ml of 1X HBSS+BSA, add it to the tube.

17 Centrifuge for 2 minutes at 339 g at 4°C

02:00:00

4 °C

18 Remove supernatant. Add 10ml 1X HBSS+BSA, re-suspend pellet. Bring up the volume to 50ml with 1X HBSS+BSA.

19 Centrifuge for 2 minutes at 339 g at 4°C

02:00:00

4 °C

20 Remove supernatant. Add 10ml 1X HBSS+BSA, re-suspend pellet. Bring up the volume to 50ml with 1X HBSS+BSA.

21 Centrifuge for 2 minutes at 339 g at 4°C

02:00:00

4 °C

22 Remove supernatant. Leave tube upside down on papertowel to drip dry (30 seconds). Wipe the interior of the tube with a Kimwipe using a long forceps (do not touch the pellet). Leave tubes upright on ice.

23 Re-suspend the pellet with 5ml of Histopaque 1077 (Sigma 1077-1 500ml) with pipette that has been rinsed with coating. Add another 5ml of Histopaque and mix using coated pipette.

24 Lay the tube at an angle on ice. Add gradually 10ml of the Mouse Media to ensure that the two mediums do not mix.

25 Centrifuge for 30 minutes at 339 g at 4°C with the **brake turned off**.

30:00:00

4 °C

Some centrifuges have an acceleration/deceleration speed setting. Use slowest rate of speed for both acceleration and deceleration .

26 Transfer the supernatant (both layers) into a new single 50ml falcon tube. Bring up to volume with 1X HBSS+BSA.

Keep tubes with the pellet on ice. If islets are not in the the supernatant, they may be in the pellet and can be recovered by hand picking in Mouse Islet Media.

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27 Centrifuge for 3 minutes at 339 g at 4°C.

03:00:00

4 °C

28 Decant the supernatant. Re-suspend the pellet in 10 ml of Mouse Media.

29 Hand pick islets if desired or necessary. Incubate islets in Mouse Islet Media at 37°C with 5% CO_2 until ready for use in experiment.

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