

Application Note

Neonatal Porcine Pancreatectomy

Objective: To surgically remove a neonatal porcine pancreas to be used in neonatal porcine islet cluster isolations.

Equipment:

- > Halothane
- > Halothane machine
- > Surgical table
- > Surgical drapes
- > Various surgical instruments
- > Sterile 4x4 gauze
- > 3-0 silk or cotton suture
- > Cobe pressure monitoring line
- > 50 ml conical tubes

Buffers:

- > Hanks Balanced Salt Solution (HBSS), warmed to 37 °C supplemented with 0.25 % bovine serum albumin, 10 mM Hepes, 100 U/ml penicillin, and 0.1 mg/ml streptomycin

Procedure:

1. Following current guidelines for animal care place 1-2 day old donor pig into surgical plain of anesthesia and prepare the abdominal area for surgery.
2. With toothed forceps and a scalpel make an incision midline to open the abdominal cavity from the sternum to just above the groin area.
3. Using an appropriate size retractor, open the incision on opposite to allow full access to the abdominal cavity.

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4. Using blunt dissection, locate and expose the abdominal aorta and vein located posterior to the renal artery and vein bifurcation.
5. Cannulate the abdominal aorta with an 18-gauge angiocath and secure the cannula with a 3-0 suture.
6. Using a pressure monitoring line, bleed the animal via the cannula into a 250 ml collection tube on ice.
7. All collected blood can be drained into the same 250 ml conical tube for later processing and serum collection (Step 12).
8. Proceed to carefully dissect the entire pancreas using retraction and blunt dissection.
9. Once the entire pancreas has been removed transfer the organ to a 50 ml conical tube containing 25 ml of HBSS.
10. Continue on to any consecutive donors while storing each pancreas in individual tubes to a maximum of four donors.
11. Once all surgeries are complete the isolation of the neonatal porcine islet clusters can commence following the protocol for islet isolation from neonatal porcine pancreases.
12. Processing of the collected blood:
 - > Put blood into blood collection tubes. (~ 10-12 ml per tube).
 - > Invert 10 times and let settle for 20 min.
 - > Centrifuge at 2000 rpm and room temperature for 10 min.
 - > Collect and save supernatant.
 - > Heat in activate serum by putting in water bath set at 58 °C for 30 min.
 - > Place in freezer when finished.

Note – Absolute care must be taken to avoid contamination during this procedure. Therefore all any sterile techniques must be followed to the letter.

The user of this protocol is solely responsible and liable.

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Application Note

Islet Isolation from neonatal Pigs with Collagenase NB 4 Standard Grade

Equipment:

- > 50 ml conical tubes
- > Chopping scissors
- > Filter holder
- > Filter mesh (500 µm)
- > Vacuum bottle
- > Pipettes
- > Large plates (4 per pancreas)

Buffers:

- > Hanks Balanced Salt Solution (HBSS), warmed to 37 °C supplemented with 0.25 % bovine serum albumin, 10 mM Hepes, 100 U/ml penicillin, and 0.1 mg/ml streptomycin
- > Ham's F-10 (warmed to 37 °C) supplemented with 10 mM glucose, 50 µM IBMX, 0.5 % bovine serum albumin, 2 mM L-glutamine, 10 mM nicotinamide, 100 U/ml penicillin, and 0.1 mg/ml streptomycin.
- > Collagenase NB 4 Standard Grade (Cat. No. S1745401) or Collagenase NB 6 GMP Grade (Cat. No. N0002779), stock solutions can be prepared and stored at -20 °C for 12 months.

Procedure:

1. Prepare the Collagenase working solution with a concentration of 0.18 PZ U/ml. For 4 pancreases weigh out the required amount of collagenase and add 100 ml HBSS. If stock solutions were prepared before dilute them accordingly with HBSS. Shake gently until dissolved and filter sterilize with a 0.22 µm filter. Place in refrigerator or on ice until ready to use.
2. Set up hood with sufficient tubes for the entire procedure, as well as the filter apparatus.
3. After surgery, take out one tube containing one pancreas at a time and wipe down with 70 % ethanol. Then place in hood.

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4. Aspirate media in each of the tubes down to 15 ml.
5. Take the first tube and chop the pancreas with sterile scissors. Bring up to 50 ml with HBSS and let settle while chopping the rest of the pancreases. Aspirate back down with a pipette (5 ml) and repeat two more times until the pancreases are all in pieces a few mm in size.
6. Bring all tubes up to 50 ml and centrifuge at 1000 rpm and room temperature for 1 min.
7. Aspirate the HBSS from the tubes and add 25 ml of the Collagenase working solution to each tube. Wrap tops with parafilm and place in water bath at 37 °C and let sit for 15 min. Then shake at about 60 rpm at 37 °C for 12 min. Hand shake the tubes for 2-5 minutes until the tissue looks sufficiently digested.
8. Remove the tubes from the bath and hand shake for another 30 seconds.
9. Wipe outside of tubes with ethanol and remove parafilm.
10. Wet a 10 ml serological pipette with HBSS. Aspirate the media up and down with a pipette until tissue is broken up and evenly suspended in the solution. Put through the filter. Wash tube 2-3 times with HBSS and put through the filter as well. Top the tube up to 50 ml with HBSS through the filter and cap.
11. Repeat for all the pancreas preparations. Pour 50 ml more HBSS through the filter into a final rinse tube.
12. Centrifuge at 1000 rpm and room temperature for 1 minute.
13. Aspirate supernatant. Resuspend the rinse tube by aspirating up and down and evenly distribute it between the different preparations. Bring each tube up to 50 ml with HBSS, ensuring that the pellet is broken up. Centrifuge for 1 minute at 1000 rpm.
14. Do a total of 3-5 washes by repeating step 13 and 14 to get rid of blood. (If you have trouble getting rid of all the blood, just bring up to speed and stop. This way, the blood stays in the supernatant and does not have time to settle.)
15. Aspirate down to pellet and re-suspend in 20 ml of Hams F-10. Add 5 ml per large non-tissue culture treated plate (use 4 dishes per pancreas).
16. Wash tube 2 times and add to one of the plates. Add a total of 35 ml media per plate with pig number.
17. Incubate at 37 °C, following the protocol for media change for neonatal porcine islets.

Ordering Information

Enzyme	Cat. No.	Pack size
Collagenase NB 4 Standard Grade	S1745402	500 mg
Collagenase NB 4 Standard Grade	S1745401	1 g
Collagenase NB 4 Standard Grade	S1745403	5 g

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