

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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