PROTOCOL FOR RAT ISLET ISOLATION

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1. Materials:

1.1 Reagents:

- Hanks balanced salt solution (HBSS, Mediatech)
- Fetal bovine serum (FBS, heat inactivated, HyClone)
- 1M HEPES Buffer (Mediatech)
- Penicillin/Streptomycin (PenStrep, Mediatech)
- DNase (Roche) [100 mg]
- Collagenase (Sigma)
- CMRL 1066 (Mediatech)
- Ficoll Gradients (1.108, 1.096, 1.037 g/cm^3, Mediatech)
- Dithizone (DTZ, Sigma)
- Dimethylsulfoxide (DMSO, Sigma)
- Isoflurane or Ketamine/Xylazine
- Betadine
- 96 % Ethanol
- 1X PBS (Mediatech)

1.2 Equipment:

1.1 Rodent Instrument Pack
- Surgical drape
- Gauze sponges
- Cotton tipped applicators
- PE 50 Polyethylene tubing
- Blunt needles 0.6mm x 25.4mm (Kendall 8881202397)
- Dissecting scissors/ Micro-dissecting scissors
- Tissue-forceps
- Micro-Mosquito forceps
- Tissue-clamp

1.2 Surgical Room Supplies
- Rodent anesthesia machine
- Carbon dioxide chamber
- Surgical microscope
- Rodent surgery board
- Sterile gloves
- Face masks
- Paper towels
- Bucket with ice
- Tape

1.3 Lab Supplies
- Water bath with shaker
- Inverted Microscope
- Centrifuge
- 5 % CO_2/O_2 tank
- 200 ml Nalgene container (normal lid and lid with tubing connectors)

- Filter bottles (500 ml, 1000 ml)
- Petri dishes/counting dishes
- 10 cc syringes
- 30 cc syringe
- 60 cc syringe
- Nalgene syringe filters (0.22µm)
- 18 ga needles
- 15 ml conical tubes
- 50 ml conical tubes
- 50 ml tube racks
- Pipet aid
- p200 pipettor with standard precision tips
- Disposable serological pipets (1, 5, 10, 25 ml)

2. Chemical Preparation:

2.1 Dissociation Buffer (25 mM HEPES /HBSS)

- Add 25 ml of 1M HEPES to 1 l of HBSS
- Sterile filter

2.2 Quenching Buffer (HBSS, 10 % FBS)

- Add 110 ml of FBS to 1 l of HBSS
- Sterilize by filtration through a 0.22 µm Nalgene filter

2.3 Culture Media (CMRL 1066, 10% FBS, PenStrep)

Combine the following chemicals:

- 1l of CMRL 1066
- 110ml of FBS
- 5ml of PenStrep
- Sterilize by filtration through a 0.22 µm Nalgene filter

2.4 Dithizone Solution (DTZ)

- Weigh out 100mg of dithizone (use a 50 ml conical tube)
- Add 10ml of DMSO
- Fill up with 40ml of 1XPBS
- Transfer solution to a 100mm Petri dish and aspirate into a 60cc syringe
- Mount to a 0.22µm syringe filter

2.5 Working Enzyme Solution (volumes/amount are stated per rat)

- Weigh out 15mg of collagenase
- Wet in 15ml of Dissociation Buffer
- Add 15µl of DNase from a [100 mg/ml working stock]
- Sterilize by filtration through a 0.22 µm Nalgene filter

3. Procedure:

3.1 Procurement:

- Gas rodent with isoflurane (3-5%) in a sealed chamber or inject Ketamine/Xylazine intraperitoneal [80/8 mg/kg].
  - For studies where insulin secretory index is to be measured the Ketamine method of anesthesia is necessary.
- Check anesthesia depth by pinching the rodent's toes
- Immediately place rodent on surgical board (head facing the surgeon) for midline incision
- Flip the abdominal organs to the left side, expose the pancreas
- Locate the pancreatic duct at the duodenum and clamp
- Flip liver above the sternum and expose the liver hilus
- Locate the common bile duct and make a small incision below the bifurcation
- Make a pointed end of one end of the PE 50 tubing
- Attach a blunt needle to the non-pointed end
- Attach a 10cc luer lock syringe (filled with 10cc of cold enzyme solution) to the needle
- Clear the tubing of air
- Place the pointed end of PE 50 tubing into the duct and gently inject the enzyme solution to distend the pancreas

- Carefully remove the pancreas and place it into a 15ml conical tube containing 5ml of cold enzyme solution and place it on ice until further processing

3.2 Pancreas Digestion:

- Combine pancreata and enzyme solution in a 200ml Nalgene container and bubble with 5%CO_2/95%O_2 for 5 minutes (at room temperature)
- Set the container in a 37°C water bath, set the shaker at 60rpm and digest for 20 minutes (max.).
- Stop enzyme activity by adding an equal volume of cold Quenching buffer and use a 30cc syringe to draw suspension up and down gently to break remaining bigger tissue.
- Filter tissue suspension through a 400μm screen.
- Using a 5ml serological pipet transfer the filtered digest into a second 200ml Nalgene container, rinse screen with Quenching buffer.
- Divide tissue suspension in 50ml conical tubes and fill up with Quenching buffer.
- Wash the tissue:
  - Spin at 1000 rpm (200g) for 1 minute (4°C), brake off
  - Aspirate supernatant
  - Resuspend pellet in 50ml of Quenching buffer
  - Repeat wash
  - Aspirate supernatant down to a dry pellet

3.3 Islet Purification:

- Resuspend the digest in 1.108 g/cm^3 Ficoll gradient at a ratio of 1:11 (tissue:Ficoll) and 12 ml is aliquoted per 50 ml conical tube
- Overlay suspension with 10 ml of 1.096 g/cm^3 and 10 ml of 1.037 g/cm^3 Ficoll

- Centrifuge the gradients at 2000 rpm for 4 minutes (4°C), brake off
- Collect islets at the interface between the upper two layers into a 50 ml conical tube containing 25 ml of Culture media
- Fill up with culture media and spin at 1000 rpm (200g) for 1 minute (4°C), brake off
- Aspirate supernatant
- Resuspend pellet in 50 ml of Culture media
- Repeat wash
- Resuspend pellet in 10 ml of Culture media
- Remove known aliquot of sample for counting

3.4 Quantification of Islets:

- Place DTZ in a grid lined counting dish
- Add known aliquot of sample
- Count the islet sample and convert into Islet Equivalents (see SOP 004)

3.5 Culture of Islets:

- Culture medium: CMRL 1066, 10 % FBS, PenStrep
- Plate the islet in a density not more than 300 IEQ per ml using non-tissue culture treated flasks
- Culture at 37°C in a 5% CO_2 incubator