

Islets isolation protocol:

- Inject mice with Nembutal (~0.05ml/adult mice) or other anesthetics;
- Perfuse pancreas (through a common bile duct) with Hanks buffer before isolation (better islets yield);
- Place pancreas into Petri dish and clean from non-pancreatic tissue;
- Transfer pancreas into 10ml glass beaker and chop it with seizers for 1.5 min;
- Place tissue into container with 13 ml of Hanks buffer and 30mg (depend on enzyme activity) of collagenase (EC 3.4.24.3 Serva, 17449);
- Digest pancreas with collagenase for 13 min at 37⁰C in water bath; while stirring with magnetic stirrer;
- Transfer digested tissue into 50 ml Falcon tube, add Hanks buffer up to 50 ml and centrifuge at ~1,000RPM to get pellet;
- Wash pellet twice, discard supernatant;
- Add Ficoll to the pellet (27%/5ml), accurately add (use syringe against the tube wall) additional 23%/3ml, 20.5/3ml, and 11% Ficoll, respectively.
- Centrifuge for 10 min at ~2,300 RPM.
- Transfer the interphase between first and second layer (count from the top) into the Falcon tube;
- Wash it with Hanks buffer;
- Transfer the pellet into Petri dish and find islets under the microscope (round shaped, white bodies).
- Spin down islets and remove excess media. Add 500 ul of Trizol, shake vigorously for 10 seconds to lyse the islets and snap free in liquid nitrogen. Store at -80°C.