

This protocol may be used to isolate islets from mouse pancreata.

**Keywords:** [mouse islets](#) [collagenase](#)

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## Reagents, supplies, and equipment:

- 37°C water bath
- collagenase P (Roche store @ -20°C)
- HBSS (Gibco #14025, store @ 4°C)
- 1 ml and 5 ml syringe
- 18G, 27G, and 30G needles
- 5.0 suture (Roboz sut-15-1 spool suture)
- BSA
- cotton swab
- Nembutal (50 mg/ml)
- 70% EtOH
- 0.9% sterile saline (for diluting Nembutol)
- BD 12 ml round bottom tubes

## Procedure: Preparation

1. Warm water bath to 37°C
2. Prepare collagenase P from 10X (12.5 mg/ml in HBSS + 0.2% BSA) stock (stored @ -80°C) in HBSS at 1.25 mg/ml. Thaw 1 - 2 vials of stock solution, depending on how many mice will be sacrificed. Each mouse requires 3.8-4 ml diluted collagenase.
3. Draw up ~3.5 ml of diluted collagenase solution into a 5ml syringe with an 18G needle and place the syringe on ice. Prepare (on ice) a separate 500 µl aliquot of the collagenase solution for dissected pancreas.
4. Bend the 30G needle under microscope to ~45° angle so that the needle bevel is facing up.
5. Ready supplies and solutions including: cotton swabs, bench paper, suture, and 0.1% BSA HBSS (add 400 µl 12.5% BSA to 50 ml HBSS).

## Procedure: Surgery

1. Inject mouse with 5 mg/ml Nembutal (20 µl/g BW) using a 1 ml syringe with a 27G needle. After about after 5-10 minutes, check to see that the mouse is fully anesthetized by pinching the feet. If there is a response, inject an additional 500 µl of Nembutal, and wait until there is no pinching response.
2. Place the anesthetized mouse on its back, spread the legs and gently tape them in an open position. Disinfect mouse abdomen with 70% EtOH.
3. Open the abdomen and locate the bile duct. Close the bile duct at the duodenum by suture ligation.
4. Remove the 18G needle from the collagenase syringe and replace it with the bent 30G needle. Inject collagenase into bile duct and inflate the pancreas.
5. Cut suture close to the knot. Separate the pancreas from the duodenum and stomach. Once exposed, remove the pancreas with the spleen attached. Remove the spleen, blot pancreas on a clean absorbent paper. Place the pancreas in a 12 ml tube containing 500 µl collagenase solution and place on ice.
6. Perform cervical dislocation on the mouse and discard the carcass.

## Procedure: Pancreas digestion and islet collection

1. Incubate pancreata at 37°C for 18 - 20 min.
2. Add ~ 5 ml cold HBSS and shake vigorously (~ 3 "shakes"), add up to 12 ml HBSS, centrifuge for 1 min at 1000 rpm.
3. Wash islets in 12 ml HBSS 3 times (same as #2 above)
4. Add 8 ml HBSS to digested pancreas and pour the digested tissue into a 10 cm plate; add an additional 4 ml HBSS to wash the tube and pour the wash into the same plate.

5. Hand pick islets under a stereoscope using a 100  $\mu$ l pipette. Transfer the picked islets to a new plate containing HBSS, and then again to a third plate of HBSS using a 10  $\mu$ l pipette to pick up islets. Place the islets in a 1.5 ml eppendorf tube. Keep the plates that contain the islets on ice when not in use.
6. Wash the islets once in 1.0 ml HBSS and carefully take out the supernatant. Freeze the pellets that contain the islets @ -80°C until ready to perform future analysis.

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