

**SERVA Collagenase NB 8 Broad Range
for the isolation of rat islets (e.g. Lewis rat islets)**

The protocol was kindly provided by:



6 Efal St.,
Kiryat Arye, Petach-Tikva
49511, Israel

Solutions:

- 1. SERVA Collagenase NB 8 Broad Range (Art. No. 17456):**
 - 1.1 Dissolve the entire content of 1 vial SERVA Collagenase NB 8 in sterile 50 mM Tris + 10 mM calcium acetate pH 7.1 to a final concentration of 12 PZ-U/ml. Mix gently and keep on ice for 30-40 min.
 - 1.2 Prepare 5 ml aliquots and store at -20 °C.

- 2. DNase I:**
 - 2.1 Store DNase I (powder) from bovine pancreas at 4 °C.
 - 2.2 Prepare 1 mg/ml DNase I solution in HBSS + 25 mM Hepes, pH 7.4.
 - 2.3 Prepare 4 ml aliquots and store at -20°C.

- 3. Glutamine:**
 - 3.1 Prepare 200 mM glutamine stock solution.

- 4. N_ω-Nitro-L-arginine methyl ester hydrochloride (L-NAME):**
 - 4.1 Prepare 50 mM stock solution with double distilled water (DDW).
 - 4.2 Prepare working solution by diluting 50 µl stock solution with 5 ml sterile DDW (1:100).
 - 4.3 Prepare 50 µl aliquots and store at -20 °C.

5. z-DEVD-FMK (Caspase-3 Inhibitor):

- 5.1 Prepare 10 mM stock solution by diluting with **ultrapure** DMSO. Store frozen at -20 °C **protected from light**.
- 5.2 Prepare 0.5 mM working solution by diluting the stock solution 1:20 in HBSS/Hepes (pH 7.4) + 2 % BSA.
- 5.3 Prepare 50 µl aliquots and store at -20 °C.

6. Comprehensive digestion solution:

- 6.1 To 30 ml HBSS/Hepes (pH 7.4) add successively:
 - 40 µl sterile 1 M CaCl₂ (final Ca⁺² concentration: 1.95 mM)
 - 5 ml SERVA Collagenase NB 8 solution (one tube, ~60 PZ-U)
 - 1 ml glutamine (200 mM)
 - 50 µl z-DEVD-FMK (one tube)
 - 50 µl L-NAME (one tube)
 - 4 ml DNase (one tube)

Based on infusion of ~9.5-10 ml/animal, the volume of the digestion solution is sufficient to process pancreata from four Lewis rats.

7. RPMI medium + RPMI/NCS medium:

- 7.1 RPMI medium: Adjust RPMI medium to pH 7.4
- 7.2 RPMI/NCS medium: RPMI medium supplemented with 10 % heat inactivated newborn calf serum (NCS)

8. Histopaque:

- 8.1 Histopaque 1.119 g/ml and 1.077 g/ml (aseptically filled)

9. Stain

- 9.1 Prepare 0.4 mg/ml Dithizone solution in DMSO (store at -20 °C).

Surgery:

1. Lightly anesthetize 4 animals simultaneously.
2. Prepare a cannula by filling a 10 ml syringe with 9.8 ml of Collagenase NB 8 solution with a dull 23 g needle attached to 10-20 cm of stiff PE50 tubing. Cut off the cannula at an angle so it resembles a needle.
3. Store cannula and extra Collagenase NB 8 solution on ice.
4. Open the abdomen and expose the pancreas as much as possible by performing a V-cut from the lower abdomen.
5. Clamp the pancreatic duct at its duodenal insertion with a hemostat, taking care not to injure the surrounding pancreatic tissue.
6. Isolate the bile duct at the proximal end, being careful not to be above the branch of the liver. If there is a lot of fat clean it off before inserting the cannula, making sure not to puncture the portal vein.
7. Cut the duct with the fine scissors at one third of the way across and insert the cannula in the duct. Make sure not to insert the cannula past the branch of the tail of the pancreas.
8. Fix the cannula in the duct by clamping it tightly with forceps and rapidly inject the Comprehensive digestion solution. The pancreas should be distended and fully dilated after ~9.85 ml of fluid injection.
9. Following the infusion of the digestion solution, carefully remove the pancreas. Start by removing from the intestine, move to the stomach and then the spleen. When the pancreas is only attached by the bile duct cut it off.
10. Place the pancreas in a 50 ml conical tube. Two pancreata are pooled in one tube.

Plan not to do more animals than surgeries that could be finished within 1 hour.

Digestion:

1. Transfer pancreata-containing tubes to a water bath pre-set at 37 °C for **~15.5 min.**
2. When incubation is over, **hand-shake** the tubes **vigorously** for ~30 s to break up the loose tissue.
3. Add 20 ml cold RPMI/NCS solution to each tube.
4. From this step on, the rest of the isolation procedure should be done on ice!
5. Wash islets with RPMI/NCS solution to remove the collagenase/DNAse. Spin down at 1100 rpm (~220 x g) for 75 s with braking.
6. Pour off the supernatant and then add ~30 ml new RPMI/NCS solution. Vortex gently (about half of maximal intensity) for 15 s and spin down as before.
7. Repeat the washing procedure for 2 more times.
8. Re-suspend the tissue homogenate in 25 ml RPMI/NCS solution and filter the suspension through a 425 µm diameter wire mesh to remove the remaining undigested tissue, fat and lymph. Remove any remaining islets from the tube and the mesh by washing with 5-10 ml RPMI/NCS solution.
9. Add 10 ml of RPMI/NCS solution and re-suspend the islets.
10. Pellet the islets by spinning once more at 1100 rpm (~220 x g) for 90 s and remove the supernatant, leaving as little excess medium as possible. This can be done by turning the tubes upside down on a paper towel.
11. Re-suspend islets in 3.5 ml Histopaque 1.077 in a 50 ml tube.

Gradient:

1. Prepare ~1.1 g/ml islet suspension by mixing 7.5 ml Histopaque 1.119 with 3.5 ml islets in Histopaque 1.077.
2. Pour 7 ml of Histopaque 1.119 under the islet phase by using a long needle.
3. Overlay islet phase with 12 ml Histopaque 1.077.
4. Pour 12 ml of RPMI-medium on top of the Histopaque gradient.
5. Spin for 20 min at 3000 rpm (~1750 x g) with very slow acceleration (2.5 min to attain full speed) and **no braking**. The centrifuge temperature should be between 5 and 10 °C.
6. Remove ~5-7 ml of the top layer that contains fat cells and cell debris.
7. Collect the islet layer from each of the interfaces by aspirating 10 ml at each interface with a disposable 10 ml serologic plastic pipette.

Final wash and gravity purification:

1. Wash islets twice with 20 ml RPMI/NCS solution at 220 x g to remove Histopaque. For the first spin use 120 s, for the second 90 s only.
2. For gravity sedimentation purification re-suspend the islets in 20 ml RPMI/NCS solution and incubate for 5 min on ice.
3. Gently remove the top 10 ml and add 10 ml of fresh NCS-medium.
4. Invert the tube for several times to distribute the islets and then allow them to sediment for 5 more minutes.
5. This process may be repeated for a total of 6 times.
6. Pellet the islets by centrifugation at 220 x g for 90 s.

Organ Culture

1. Use CMRL:RPMI 1:1 + 10 % FCS (complete CR; 8.34 mM glucose) as culture medium.
2. If long term (>7 days) cultivation is requested:
Seed islets at a density of 600-700 per 90 mm **bacterial grade** Petri dish. Cultivate islets for the first three days at 27 °C.