In partnership with Nordmark Biochemicals

amsbio

Application Note

Islet Isolation from Rats with Collagenase NB 8 Broad Range

Solutions:

Collagenase NB 8 solution:

Dissolve Collagenase NB 8 Broad Range in sterile 50 mM Tris buffer + 10 mM calcium acetate, pH 7.1, to a final concentration of 12 PZ U/ml. Keep the solution on ice for 30-40 min for complete dissolution of the collagenase. Prepare 5 ml aliquots and store at -20 °C.

DNase I solution:

Dissolve DNase I in HBSS + 25 mM Hepes, pH 7.4, to a final concentration of 1 mg/ml. Prepare 4 ml aliquots and store at -20 °C.

N_{ω}-Nitro-L-arginine methyl ester hydrochloride (L-NAME):

Prepare a 50 mM stock solution with double distilled water (DDW). For preparing the working solution dilute 50 µl stock solution with 5 ml sterile DDW (1:100). Store 50 µl aliquots at -20 °C.

z-DEVD-FMK (Caspase-3 inhibitor):

Prepare a 10 mM stock solution by diluting 1 mg with 145 µl ultrapure DMSO. Store at -20 °C protected from light. Prepare the working solution (0.5 mM) by diluting the stock solution 1:20 in HBSS/Hepes (pH 7.4)+ 2 % BSA. Store 50 µl aliquots at -20 °C.

Digestion solution:

Add successively to 30 ml HBSS/Hepes, pH 7.4

40 µl sterile 1 M CaCl2 (final Ca+2 concentration: 1.95 mM)
5 ml Collagenase NB 8 solution (approx. 60 PZ U)
1 ml 200 mM glutamine
50 µl z-DEVD-FMK working solution
50 µl L-NAME working solution
4 ml DNAse I solution

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

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Solutions (continued):

- RPMI medium: Adjust RPMI medium to pH 7.4
- RPMI/NCS medium: RPMI medium supplemented with 10 % heat inactivated newborn calf serum
- Histopaque 1.119 g/ml and 1.077 g/ml (aseptically filled)
- Dithizone 0.4 mg/ml dissolved in DMSO (store aliquots at -20 °C)
- Organ culture medium:
  CMRL medium : RPMI medium 1 : 1 + 10 % FCS (total glucose concentration: 8.34 mM)

Surgery:

1. Lightly anesthetize 4 animals simultaneously.
2. Prepare a cannula by filling a 10 ml syringe with 9.8 ml of digestion solution with a dull 23 g needle attached to 10-20 cm of stiff PE50 tubing. Cut off the cannula at an angle to resemble a needle.
3. Store cannula and extra digestion solution on ice.
4. Open the abdomen and expose the pancreas as much as possible by making a V-cut from the lower abdomen.
5. With the use of hemostat, clamp-off the pancreatic duct at its duodenal insertion, 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. Hold the cannula in the duct by clamping it tightly with forceps and rapidly inject the digestions solution. The pancreas should be distended and fully dilated after injecting approx. 9.85 ml solution.
9. Following the infusion of the digestion solution, carefully remove the pancreas. Start by removing from the intestine, moving 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 to do no more animals than surgeries that could be finished within 1 hour!

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Digestion:

1. Incubate all tubes with pancreata in a water bath (37 °C) for ~15 to 18 min (optimal digestion time should be determined for each lot of collagenase by monitoring the digestion process).
2. When incubation is over, hand-shake the tubes vigorously for ~30 s to break up the 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 and DNase. Centrifuge at 1100 rpm (~220 x g) for 75 s with brakes on.
6. Pour off the supernatant and add ~30 ml RPMI/NCS solution. Vortex gently (about half of maximal intensity) for 15 s and centrifuge (see step 5).
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 centrifugation 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 a ~1.1 g/ml islet suspension by mixing 7.5 ml of Histopaque 1.119 with 3.5 ml of islets in Histopaque 1.077.
2. Pour 7 ml of Histopaque 1.119 under the islet phase by using a long needle.
3. Overlay the islet phase with 12 ml Histopaque 1.077.
4. Pour 12 ml of RPMI medium on top of the Histopaque gradient.
5. Centrifuge 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 °C 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.

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Final wash and gravity purification:

1. Wash the islets twice by adding 20 ml RPMI/NCS solution and centrifugation at 220 x g to remove the Histopaque. Centrifuge for 120 s in the first washing step and for 90 s in the second washing step.

2. For purification by gravity sedimentation re-suspend the islets in 20 ml RPMI/NCS solution and incubate for 5 min on ice.

3. Gently remove 10 ml from the top and add 10 ml of fresh RPMI/NCS medium.

4. Invert the tube for several times to redistribute the islets and allow the islets to sediment for 5 min.

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.

7. If long term cultivation (>7 days) is requested, seed islet with a density of 600-700 per 90 mm Petri dish.

Ordering Information

Product	Cat. No.	Pack size
Collagenase NB 8 Broad Range	S1745601	250 mg
	S1745602	1 g

This protocol was developed by:

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