Calculating beta cell mass

This protocol may be used to harvest, fix, embed, and immunostain mouse pancreas and perform morphologic calculations of mouse islets and beta cells.

Keywords: embedding, fixation, immunohistochemistry, parafin embedding

Expand

Reagents, supplies, and equipment: *Fixation*

- surgical instruments for pancreas dissection
- 4% PFA
- rotating "Nutator"
- 70% EtOH
- paraffin
- microtome

Procedure: *Fixation*

1. Weigh mouse
2. Euthanize mouse utilizing CO_2 and immediately remove the pancreas
3. Weigh the pancreas (dry)
4. Fix in 4% PFA for 4 hr at 4°C on a rotating Nutator
5. Wash 2 x 15 min in 1X PBS at 4°C on a rotating Nutator
6. Incubate O/N at 4°C in 70% EtOH
7. Dehydrate
8. Embed in paraffin
9. Starting in the middle of the block, section pancreas in 5 µm section placing 5 sections on each slide. Prepare 100 slides (or 500 sections).
10. Select every 10^{th} slides for immunohistochemistry. There should be at least 250 µm between immunostained sections.
11. Select one section per slide for IHC cell mass calculations.

Reagents, supplies, and equipment: *Immunohistochemistry*

1. PBS
2. PBS-T (0.2% Tween 20 in PBS)
3. xylene
4. 100%, 95%, 70% EtOH, MeOH and 30% H_2O_2 in water.
5. Dako Cytomation pen
6. Vectastain Elite ABC kit (Standard*)
7. DAB Peroxidase Substrate kit (Vector)
8. eosin
9. Nikon scanner
10. Metamorph software

Procedure: *Immunohistochemistry*

1. De-paraffin in a fume hood using slid jars/holders
2. Submerge 2 x 5 min in xylene
3. Submerge 2 x 5 min in 100% EtOH
4. Submerge 1 x 5min in MeOH
5. Incubate 1 x 30 min in MeOH/Peroxide (100 ml MeOH + 4ml 30% H_2O_2)
6. Submerge 2 x 5 min in 95% EtOH
7. Submerge 1 x 5 min in 70% EtOH
8. Submerge 1 x 5 min in tap H_2O

9. Submerge 3 x in PBS
10. Circumscribe the section using a Pap pen
11. Block by incubating sections in 1% BSA + 5% NS (normal serum) in PBS-T for 1hr X 30 min at RT.
12. Incubate sections with anti-insulin antibody at 1:1000 in 1% BSA + 5% NDS in PBS-T O/N at 4 °C.
13. Wash sections 2 x 5 min in PBS-T, then 1 x 5 min in PBS.
14. Incubate sections with biotin coupled secondary antibody for 30 min at RT.
15. Wash 2 x in PBS
16. Using the reagents from the Vectastain kit, incubate sections with the Avidin-HRP (1 ml PBS + 10 µl solution A [Avidin DH] + 10 µl solution B [biotinylated enzyme]) for 30 min at RT (prepare the A + B complex at least 30 min prior to incubation).
17. Wash 2 x with the appropriate buffer.
18. Prepare DAB with reagents from Vector DAB kit as follows: 5 ml DI H_2O + 2 drops buffer mix + 4 drops DAB mix + 2 drops H_2O_2)
19. Incubate with the proper substrate and monitor the staining under the microscope until color is at the proper intensity.
20. Wash 2 x 5 min in H_2O
21. Counter stain with eosin (dip < 15 sec)
22. Incubate 2 x 5 min 70% EtOH
23. Incubate 2 x 5 min 95% EtOH
24. Incubate 2 x 5 min 100% EtOH
25. Dip in xylene and mount with the Permount (Vector).

If secondary antibody is linked to HRP incubate sections with HRP coupled secondary antibody and omit steps 16 and 17.

26. Chose sections in the same position on each slide (for instance, 2nd section in every slide).
27. Scan the entire section on the Nikon scanner
28. Erase the "noise" on the image (such as adipose tissue or lymph nodes, etc.).
29. Open the image (minus noise) in Metamorph and measure the entire area of the pancreas (eosin stained).
30. Next, measure the stained islet area (brown) . Export the data into an Excel spreadsheet to calculate the ratio of stained islet to whole pancreas.
31. Perform these same measurements on 10 sections and calculate the average ß cell mass by multiplying the islet:pancreas area ratio X the pancreatic weight.