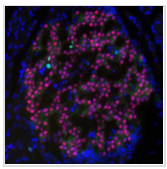

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Immunofluorescent staining of pancreatic sections

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This protocol describes the steps for performing fluorescence immunohistochemistry on fixed-frozen pancreatic tissue sections. It is suitable for pancreatic tissue isolated from rats and mice at postnatal to adult stages. We routinely apply this protocol to quantify total and proliferating or apoptotic beta-, alpha- and delta-cells. Briefly, pancreata are fixed in 4% paraformaldehyde solution and cryoprotected overnight in 30% sucrose. Tissue are then embedded, frozen, sectioned and mounted on slides. Antigen retrieval is performed using sodium citrate buffer prior to immunostaining.

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Glucose and fatty acids synergistically and reversibly promote beta cell proliferation in rats. Moullé VS, Vivot K, Tremblay C, Zarrouki B, Ghislain J, Poitout V. Diabetologia. 2017 May;60(5):879-888. doi: 10.1007/s00125-016-4197-8. Epub 2017 Jan 11. PMID: 28078385.
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immunohistochemistry, pancreas

protocol ,

Caroline Tremblay

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37812

The slides should not be allowed to dry during the staining steps. Drying out will cause nonspecific antibody binding and therefore high background staining.

MATERIALS

☒ Hoechst 33342, Trihydrochloride, Trihydrate - 10 mg/mL Solution in Water **Invitrogen - Thermo Fisher Catalog #H3570**

☒ OCT (Optimal Cutting Temperature compound) **Sakura Finetek Catalog #4583**

☒ VECTASHIELD® Hardset™ Antifade Mounting Medium **Vector Laboratories Catalog #H-1400**

☒ Phosphate Buffered Saline 10x (solution) **Bio Basic Inc. Catalog #PD8117**

☒ Paraformaldehyde 10% buffered **Newcomer Supply Catalog #13301A**

☒ Donkey serum **Sigma Aldrich Catalog #S30-100ML**

☒ Triton X-100 **Sigma Aldrich Catalog #T9284**

☒ Dako Pen Delimiting pen **Agilent Technologies Catalog #S2002**

☒ Bovin serum albumin **Sigma Aldrich Catalog #A7888**

☒ Sucrose Ultra Pure **Bioshop Catalog #SUC507.5**

☒ Sodium citrate crystals reagent **ACP Chemicals Catalog #S2990**

☒ Superfrost Plus Microscope Slides **Fischer Scientific Catalog #12-550-15**

☒ MilliQ water **Contributed by users**

Surgipath® Clear Disposable Base Molds
Leica 75809-376

Well, 250ml. w/ lid, green, xylene resistant
TBS SS-WLG

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When working with PFA, always work in the chemical hood.

Make sure you have the right combination of secondary and primary antibodies with regards to the host species and the fluorophore.

Preparation of cryosections 1d

1 Tissue fixation

19h

When working with PFA, always work in a chemical hood.

Harvest the pancreas and place it in a 50 ml Falcon tube containing **30 mL** of cold 4 % PFA (**12 mL** of 10 % PFA + **18 mL** of PBS).

Fix for **04:00:00** at **4 °C** in the dark.

Then, working in a chemical hood, delicately remove the pancreas with forceps and place it on brown paper to absorb the fixative.

Place the organ in a new 50 ml Falcon tube containing **30 mL** of a 30 % sucrose solution (**9 g** of sucrose and **30 mL** of PBS).

Store **15:00:00** at **4 °C** in the dark.

The next day, delicately remove the pancreas with forceps and place it on brown paper to absorb the sucrose solution. Place it in a mold, cover with OCT and freeze at **-80 °C** until ready for sectioning.

2 Preparing cryosections

2h

Set the cryostat and the pedestal temperature at **-20 °C** .

Gather all of the needed material (OCT, slides, pencil, blades, paintbrushes, aluminium foil, slide box, tissues).
Cut cryosections at 0.8 µm thickness and collect on Superfrost Plus microscopic slides.

Store the sections at **-80 °C** until ready for staining.

Immunofluorescent staining 1d

3 Antigen retrieval

1h

Bring the slides to **Room temperature** by putting them in the slide holder in PBS until ready to perform antigen retrieval step.

Put the holder in a 250 ml well filled with milliQ water for **00:05:00** at **Room temperature** .

Transfer the slides to the sodium citrate solution (**10 millimolar (mM)** **6**).

Heat the slides in the microwave with the following sequence:

Heat **00:03:00** (if the solution starts to boil, stop heating and add the remaining time to the pause section; the antigen retrieval step must last a total of **00:20:00**).

Remove from the microwave and let stand **00:17:00** .

Heat **00:01:00** and let cool down for **00:30:00** at **Room temperature** .

Rinse the slides in milliQ water for **00:05:00** .

Transfer slides to PBS for **00:05:00** .

4 Blocking

1h

Dry the slides and outline the tissue with a hydrophobic barrier pen.

Place the slides with the tissue facing up in an humidified chamber and add a sufficient volume of blocking solution (**0.1 % (v/v)** Triton, **1 % (v/v)** BSA plus **5 % (v/v)** normal donkey serum in PBS). Use between **150 µL** - **300 µL** depending on the size of the tissue.

Block for **01:00:00** at **Room temperature** .

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5 Primary antibody staining

15h

> At no time should the slides be allowed to dry. Drying out will cause nonspecific antibody binding and therefore high background staining.

Remove the blocking solution and replace with **150 µL** - **300 µL** antibody mix diluted in blocking solution.

Incubate in the humidified chamber **15:00:00** at **4 °C** .

6 Secondary antibody staining

1h 30m

The following morning decant the primary antibody mixture and wash the slides three times in PBS for **00:05:00** using a slide holder and well.

Return slides to the humidified chamber and incubate with the secondary antibody diluted in blocking solution for **01:00:00** at **Room temperature** .

Remove the antibody mixture and wash three times **00:05:00** in PBS.

7 Hoechst staining and mounting

30m

Label nuclear DNA by incubating the slides in the Hoechst solution (**10 µL** of Hoechst in **250 mL** of PBS) for **00:10:00** at **Room temperature** using slide holder and well.

Wash the slides three times **00:05:00** in PBS.

Mount slides using VECTASHIELD HardSet Mounting Medium without DAPI.

Place the mounted slides in a cardboard slide tray holder in the dark until ready to acquire the images with a fluorescent microscope.

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