Immunohistochemical staining of insulin and glucagon in islets of formalin-fixed human pancreas

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Abstract

Insulin and glucagon hormones were detected in paraffin sections (4 µm thickness) of formalin-fixed human pancreases. Insulin and glucagon were detected immunohistochemically using mouse anti-human insulin mAb (ascites; Sigma) and mouse anti-porcine glucagon mAb (ascites; Sigma), respectively, with biotinylated anti-mouse IgG and avidin-biotin-complex (ABC reagent; PK-2200, Vector Labs). Background staining was checked using the corresponding isotype control Ig instead of the primary antibody. 3-amino-9-ethylcarbazole (AEC) was used as the chromogen. Stained sections were imaged using a light microscope with attached camera (Olympus BX41).

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Before start

Materials:

1. Prepare graded alcohols and xylene for deparaffinizing tissue sections: 2 x xylene (250 ml/slide container), 2 x absolute ethanol (250 ml/slide container), 1 x 90% ethanol (250 ml), 1 x 70% ethanol (250 ml).

2. Prepare acetate buffer components:

(i) 0.1N acetic acid: 290 µl glacial acetic acid in 50 ml deionized water

(ii) 0.1M sodium acetate: 410 mg anhydrous CH_3COONa in 50 ml deionized water.

Prepare 0.1M acetate buffer (pH 5.2) by mixing 10.5 ml 0.1N acetic acid and 39.5 ml 0.1M sodium acetate.

3. Prepare 18% (w/v) sodium azide.

4. Prepare stock solution of 3-amino-9-ethylcarbazole (AEC; chromogen, 8 mg/ml: 40 mg AEC in 5 ml N-N-dimethyl formamide; protect from light and refrigerate at 4°C.

5. Prepare M.O.M. diluent: 200 µl M.O.M. protein concentrate stock solution (Immunodetection kit, PK-2200, Vector) in 2.5 ml phosphate-buffered saline (PBS) for use either as a blocking step to minimize non-specific Ig binding or for diluting antibodies.

6. Mabs and pAbs:

Mouse anti-human insulin mAb (ascites), Sigma-Aldrich #12018

Mouse anti-porcine glucagon mAb (ascites), Sigma-Aldrich #G2654

Biotinylated anti-mouse IgG, M.O.M immunodetection kit, Vector Labs # PK-2200

Mouse IgG_{1K}, eBioscience # 14-4714-85

7. Other reagents:

Hydrogen peroxide (30% w/w), Chem-Supply Pty Ltd (Australia) #HA154-500M
Methanol, Merck #106009
Sodium azide, Sigma-Aldrich #S2002
3-Amino-9-ethylcarbazole (AEC), Sigma-Aldrich #A5754
Animal free blocker, Vector Labs #SP-5030
Stock protein concentrate, M.O.M immunodetection kit, Vector Labs # PK-2200
Avidin-biotin complex (ABC), M.O.M immunodetection kit, Vector Labs # PK-2200
N-N-dimethyl formamide, Sigma #D158550
Glycergel mounting medium, Dako #C0563

Protocol

Step 1.

See Guidelines, "Before starting"

Step 2.

Deparaffinize slides in each xylene for 1 min. rehydrate slides in graded alcohols beginning in absolute ethanol (10 dips)/ container of absolute ethanol), followed by 90% ethanol (10 dips) and 70% ethanol (10 dips). Wash well in running tap water for 5 min.

Step 3.
Wash 2 x 2 min in 250 ml phosphate-buffered saline (PBS).

Step 4.
Wipe around sections with a tissue, encircle the sections using a diamond pencil and place in clean 250 ml slide container of fresh PBS. Wipe away excess PBS around each section using a tissue.

Step 5.

Block endogenous peroxidase activity by incubating sections in 3% hydrogen peroxide in methanol (peroxidase block working solution) for 5 min. For peroxidase block working solution add 10 µl hydrogen peroxide to 10 ml methanol; take 5ml of 3% H_2O_2/methanol and add 28 µl 18% (w/v) sodium azide.

Step 6.
Wash off peroxidase block using PBS and transfer slides to a clean 250 ml slide container of fresh PBS. Wash slides 3x over 10 min.

Step 7.
Dilute primary antibodies in diluted stock protein concentrate (diluent prepared from M.O.M. Immunodetection kit).

Step 8.
Wipe around sections using tissue to remove excess PBS.

Step 9.

Apply:

(i) 1/500 dilution of anti-insulin mAb (stock 20 mg/ml) or 40 µg/ml mouse IgG_{1K} (as isotype control), diluted in M.O.M. diluent, 125-150 µl/section at room temperature for 30 min.

or

(ii) 1/500 dilution of anti-glucagon mAb (stock 31 mg/ml) or 62.5 µg/ml mouse IgG_{1K} (as isotype control), diluted in M.O.M. diluent, 125-150 µl/section at room temperature for 30 min.

Step 10.
Wash off primary antibody with PBS and transfer slides to slide container with 250 ml PBS. Wash 2 x 2min.

Step 11.

Wipe around sections using tissue and incubate with 1/250 diluted secondary biotinylated-anti-mouse IgG (M.O.M immunodetection kit), 150 µl/section, for 10 min at room temperature.

Step 12.
Wash off secondary antibody with PBS and transfer to slide container with 250 ml PBS. Wash slides 2 x 2min.

Step 13.
Wipe around sections using tissue and cover with Vectastain ABC reagent (M.O.M immunodetection kit), for 5 min at room temperature.

Step 14.
Wash off ABC reagent with PBS and transfer to slide container with 250 ml PBS. Wash slides 3x in 10 min.

Step 15.

Prepare AEC working solution: 4.75 ml acetate buffer (see Guidelines), 0.25ml AEC stock solution and 25 µl 3% H_2O_2. Filter using a disposable 0.2 µm filter. Use within 2 hours of preparation, refrigerate for short-term storage. Protect from light.

Step 16.
Wipe around sections using tissue and cover the sections with AEC solution for 30 min at room temperature.

Step 17.
Wash off AEC solution with deionized water and transfer slides to slide container with 250 ml deionized water. Wash 3x in 10min.

Step 18.

Lightly counterstain with Gill’s hematoxylin, wash in deionized water (2 x) and briefly dip in ammonium water (100 µl ammonia in 250 ml deionized water), 2 x 2 sec. Wash in deionized water (2 x in 250 ml) and coverslip using glycergel mounting medium.

Step 19.
Photograph sections using a light microscope with camera attachment.