

Direct Immunohistochemical Staining of Insulin in Paraffin Embedded Tissue Sections

INTRODUCTION

Immunohistochemical staining is performed with an enzyme conjugated ligand and a chromogenic substrate that is catalyzed by the enzyme to a colored precipitate. The technique can be used on frozen and paraffin embedded tissue sections and on cytological samples. The immunohistochemically stained sample is analyzed with a light microscope and the method is used for analysis of protein localization in a tissue or in a cell.

The HRP conjugated Anti-Insulin Affibody[®] molecule is a specific affinity ligand that can be used for convenient, direct immunohistochemical staining of insulin in paraffin embedded tissues sections of human, rat and mouse origin. Since staining with HRP conjugated Anti-Insulin Affibody[®] molecule is done in a single step, the process is completed in 1 hour.

RESULTS OF IMMUNOHISTOCHEMICAL STAINING OF PARAFFIN EMBEDDED TISSUE SECTIONS IN HUMAN PANCREAS

Sections of paraffin embedded human pancreases were used for immunohistochemical staining with a HRP conjugated Anti-Insulin Affibody[®] molecule. The tissue sections were stained for 45 minutes at room temperature, developed with DAB substrate and counter stained with Mayers Haematoxylin. The resulting microscope image shows strong brown membrane staining of islet cell cells in the pancreas leaving the rest of the pancreatic tissue negative. Thus, the HRP conjugated Anti-Insulin Affibody[®] molecule is a rapid reagent for insulin specific immunohistochemical staining of paraffin embedded tissues.

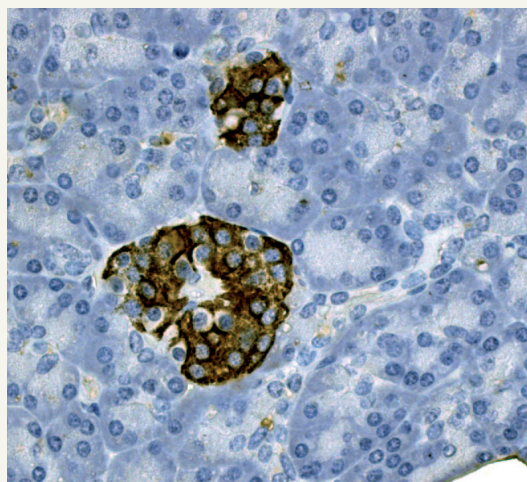


Fig. 1. Paraffin embedded human normal pancreas was stained with HRP conjugated Anti-Insulin Affibody[®] molecule. Strong cytoplasmic staining was observed in islet cells but not in surrounding tissues.

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MATERIALS AND BUFFERS REQUIRED

Staining reagent: Anti-Insulin Affibody[®] molecule, HRP conjugated (Affibody cat no 10.0817.05.0002)

Xylen: (Histolab cat no 02080)

Ethanol

Sodium citrate buffer: 10 mM Sodium citrate, pH 6.0

Staining dishes

PBS: 2.68 mM KCl, 1.47 mM KH₂PO₄, 137 mM NaCl, 8.1 mM Na₂HPO₄, pH 7.4

Formaldehyde

Hydrogen Peroxide (H₂O₂): 3% in PBS

Substrate: DAB (Dako cat no 3465)

Mayers Haematoxylin: (Histolab cat no 1820)

Mounting medium: Pertex (Histolab cat no 00814)

ANTIGEN RETRIIVAL

1. Deparaffinize paraffin embedded sections of the tissue in 2 changes of xylene, 4 minutes each.
2. Hydrate in 2 changes of 100% ethanol for 4 minutes each, followed by changes in 95% and 80% ethanol for 2 minutes each. Then rinse in distilled water for 2 minutes.
3. Immerse the slides in a staining dish filled with sodium citrate buffer. Use a microwave oven to heat/boil the slides for 2 x 5 minutes. Cool the slides by rinsing in room temperature water. Alternatively, pre-heat a steamer or water bath with staining dish containing sodium citrate buffer or citrate buffer until the temperature reaches 95-100°C. Immerse the slides in the staining dish, place the lid loosely and incubate for 20-40 minutes (optimal incubation time should be determined by user). Turn off the steamer or water bath, remove the staining dish to room temperature and allow the slides to cool for 20 minutes.
4. Let the tissue sections stand in PBS for 7 minutes.

STAINING PROTOCOL

1. Blocking: endogenous peroxidase should be blocked by incubation in 3% hydrogen peroxide (H₂O₂) for 15 minutes. Rinse the slide in PBS before and after blocking. The need for further blocking should be determined by the user. After blocking, remove blocking solution and make sure that the surface is dry around the tissue section.
2. Add the HRP conjugated Anti-Insulin Affibody[®] molecule, diluted in PBS. Make sure that the added volume completely covers the tissue. A final dilution between 1:50 – 1:150 of the conjugated Affibody[®] molecule is recommended. The user is required to determine the optimal concentration.
3. Incubate in a moist chamber for 45 minutes at room temperature.
4. Wash slides gently with PBS, approximately 2 x 5 minutes in a staining dish.
5. Add DAB substrate and develop for approximately 7 minutes. The time may differ depending on the substrate and the user is recommended to determine optimal developing time.
6. Rinse in a staining dish under tap water for 3 minutes.
7. For contrast, stain the nuclei with Haematoxylin for approximately 20 seconds and rinse under tap water in a staining dish for 10 minutes.
8. Dehydrate the slide by immersing the slides in 80, 95 and 100% ethanol.
9. Mount the slide with mounting medium.

LIMITATIONS

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