

Direct Immunohistochemical Staining of Insulin of Frozen 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 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 frozen tissues sections of human, rat and mouse origin. Since staining with HRP-conjugated Anti-Insulin Affibody[®] molecule is a single step, the process is completed in less than 1 hour.

RESULTS IMMUNOHISTOCHEMICAL STAINING OF FROZEN TISSUE SCETIONS OF RAT PANCREAS

Rat pancreases were snap-frozen in liquid nitrogen, sectioned and used for immunohistochemical staining with the HRP-conjugated Anti-Insulin Affibody[®] molecule. The frozen tissue sections, previously fixed with formaldehyde, were stained with HRP-conjugated Anti-Insulin Affibody[®] molecule for 45 minutes at room temperature. The staining was developed with DAB substrate and the tissue sections were counter stained with Mayers Haematoxylin. The resulting microscope image shows strong cytoplasmic 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 frozen tissue sections.

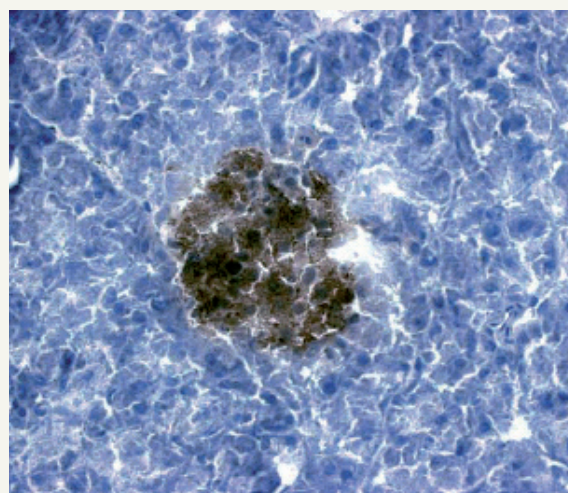


Fig. 1. Detection of insulin producing islet cells in frozen tissue sections of rat pancreas using HRP-conjugated Anti-Insulin Affibody[®] molecule.

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MATERIALS AND BUFFERS REQUIRED

Staining reagent: HRP conjugated Anti-Insulin Affibody[®] molecule (Affibody cat no 10.0814.05.0005)

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

Substrate: DAB (Dako cat no 3465)

Mayers Haematoxylin: (Histolab cat no 1820)

Hydrogen Peroxide (H₂O₂): 3% in PBS

Mounting medium: Pertex (Histolab cat no 00814)

Formaldehyde

Staining dish

PROTOCOL

1. Use 4-6 µm thick sections of frozen tissues.
2. Thaw slides for 10-15 minutes at room temperature.
3. Fix the tissue section with 3% formaldehyde in PBS for at least 10 minutes at room temperature.
4. Wash the slides gently in a staining dish with PBS, 2 x 1 minute before staining.
5. Blocking: endogenous peroxidase should be blocked by incubation in hydrogen peroxide (H₂O₂) for 15 minutes. Rinse the slide in PBS before staining. The need for further blocking should be determined by the user. Remove blocking solution and make sure that the surface is dry around the tissue section.
6. 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.
7. Incubate in a moist chamber for 45 minutes at room temperature.
8. Wash slides gently with PBS, approximately 2 x 5 minutes in a cuvet.
9. 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.

LIMITATIONS

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