APPLICATION NOTE

Direct Immunohistochemical Staining of HER2 of Frozen Tissue Sections

INTRODUCTION

Immunohistochemical staining is performed with an enzyme conjugated ligand and a 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-HER2 Affibody® molecule is a highly specific affinity ligand that can be used for convenient, direct immunohistochemical staining of HER2 receptors in frozen tissues sections of human origin. Cross-reactivity to other species has not been tested. Since staining with HRP-conjugated Anti-HER2 Affibody® molecule is a single step, the process is completed in less than 1 hour. The use of HRP-conjugated Anti-HER2 Affibody® molecule on paraffin embedded tissue sections is not recommended.

RESULTS

IMMUNOHISTOCHEMICAL STAINING OF FROZEN TISSUE SECTIONS

Xenograft tumors of the human ovarian adenocarcinoma, SK-OV-3 was soaked in formaldehyde and then snap-frozen in liquid nitrogen and used for immunohistochemical staining with the HRP-conjugated Anti-HER2 Affibody® molecule. Frozen tissue sections were stained with HRP-conjugated Anti-HER2 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 brown membrane staining of tumor cells in the xenograft whereas the mouse connective tissue that surrounds and traverses the tumor remains negative. Thus, the HRP-conjugated Anti-HER2 Affibody® molecule is a rapid reagent for HER2 specific immunohistochemical staining of frozen tissue sections.

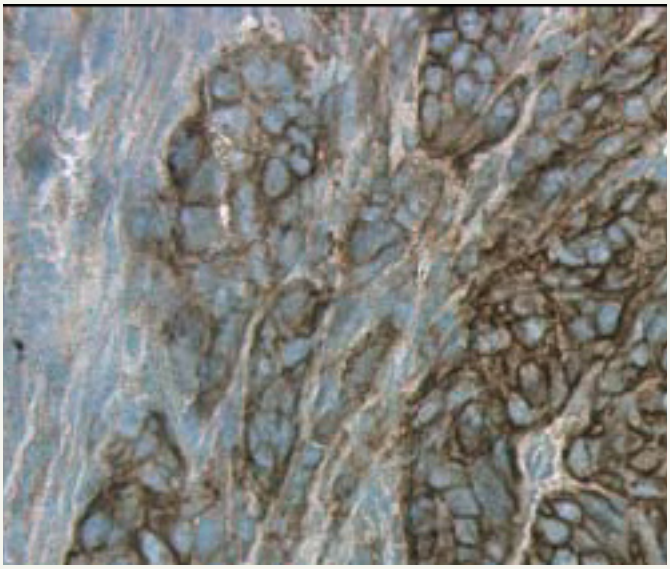

Fig. 1. Frozen tissue sections of a SK-OV-3 xenograft tumor was stained with HRPconjugated Anti-HER2 Affibody® molecule. Strong membrane staining was observed in tumor cells but not in mouse conncective tissues.

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MATERIALS AND BUFFERS REQUIRED

Staining reagents: HRP conjugated Anti-HER2 Affibody® molecule (Affibody cat no 10.0817.05.0001)
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%
Mounting media:
Formaldehyde:

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 cuvet 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-HER2 Affibody® molecule, diluted in PBS. Make sure that the added volume completely covers the tissue. A final dilution between 1:250 – 1:2000 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

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