The protocols of immunohistochemistry Staining

In Frozen Tissue Arrays

DAKO IHC Protocols are recommended for US Biomax tissue (array) sections.

Frozen tissue array is recommended be fixed in 10% para-formaldehyde solution for 10 min at room temperature before the IHC steps even the arrays were fixed in ethanol or briefly in cold acetone before.

GENERAL CSA PROCEDURE FOR USE WITH MONOCLONAL MOUSE PRIMARY ANTIBODIES.

1. Incubate tissue 5 minutes with peroxidase blocking reagent (optional).
2. Rinse with and place 3–5 minutes in buffer.
3. Incubate 5 minutes with a protein-blocking reagent to reduce background.
4. Tap off excess protein block. DO NOT RINSE. Incubate 15 minutes with each of the following 5 reagents, repeat Step 2 after each:
5. Primary mouse antibody (or negative control reagent).
6. Biotinylated rabbit anti-mouse link antibody.
7. Streptavidin-biotin complex.
8. Amplification reagent.
9. Streptavidin-peroxidase complex.
10. Incubate 5 minutes with substrate-chromogen solution.
11. Rinse with distilled water.
12. Counterstain with hematoxylin (optional) and coverslip.

GENERAL LSAB PROCEDURE (HRP) FOR USE WITH MONOCLONAL MOUSE PRIMARY ANTIBODY

1. Incubate 5 minutes with peroxidase blocking reagent (optional).
2. Rinse with and place 3–5 minutes in wash buffer. Incubate 10 minutes with each of the following 4 reagents; repeat Step 2 after each:
3. Primary antibody (or negative control reagent).
4. Biotinylated link antibody.
5. Streptavidin-HRP.
6. Substrate-chromogen solution.
7. Counterstain with hematoxylin (optional) and coverslip.

CHAIN POLYMER-CONJUGATED TECHNOLOGY

This technology (DAKO EPOSTM and DAKO EnVision Systems) is protected by DAKO patents

GENERAL EPOS PROCEDURE (PEROXIDASE)

1. Quench for endogenous peroxidase activity (optional).
2. Rinse with and place 3–5 minutes in wash buffer.
3. Incubate 10–60 minutes with EPOS conjugate.
4. Repeat Step 2.
5. Incubate 5–15 minutes with substrate-chromogen
6. Counterstain (optional) and coverslip.

GENERAL ENVISION PROCEDURE (PEROXIDASE)

1. Incubate 5 minutes with peroxidase blocking reagent (optional).
2. Rinse with and incubate 3–5 minutes in wash buffer.
3. Incubate with primary antibody for 10* minutes.
4. Repeat Step 2.
5. Incubate for 5–10 minutes in polymer solution.
6. Repeat Step 2 twice.
7. Incubate 5–10 minutes in substrate-chromogen.
8. Repeat Step 2.
9. Counterstain and coverslip.

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