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IHC Protocol using Proteinase K based Antigen Retrieval

Immunohistochemistry protocol for formalin-fixed, paraffin embedded tissues

Tissue Sectioning, Deparaffinization, and Rehydration

1. Section paraffin blocks into 4 micron sections with microtome and place on charged microscope slides (Fisher, ProbeOn, Cat. #22230900).
2. Heat slides in a tissue-drying oven for 45 minutes at 60°C.
3. Wash slides in 3 changes of xylene for 5 minutes each at room temperature.
4. Wash slides in 3 changes of 100% alcohol for 3 minutes each at room temperature.
5. Wash slides in 2 changes of 95% alcohol for 3 minutes each at room temperature.
6. Wash slides in 1 change of 80% alcohol for 3 minutes at room temperature.
7. Rinse slides in running distilled water for 5 minutes at room temperature.

The following steps are to be conducted at room temperature. Do not allow tissues to dry at any time during the staining procedure.

Antigen Retrieval

1. Rinse slides in 1X TBS with Tween (TBST) for 1 minute.
2. Apply a working solution of Proteinase K (DAKO, Cat. #S3020) to the slides and incubate for 10 minutes.
3. Rinse slides in 1X TBST for 1 minute.

Immunostaining with AP-Vector Red Detection System

1. Apply Universal Protein Block (DAKO, Cat. #X0909) to the slides and incubate for 20 minutes.
2. Drain protein block from slides.
3. Apply diluted primary antibody to the slides and incubate for 45 minutes.
4. Rinse slides in 1X TBST for 1 minute.
5. Apply a biotinylated secondary antibody to the slides (specific to the host of the primary antibody) and incubate for 30 minutes.
6. Rinse slides in 1X TBST for 1 minute.
7. Apply Alkaline Phosphatase Streptavidin (Vector, Cat. #VEC-AK-5000) to the slides and incubate for 30 minutes.
8. Rinse slides in 1X TBST for 1 minute.
9. Apply Alkaline Phosphatase Chromogen Substrate (Vector, Cat. #VEC-AK-5000) to the slides and incubate for 30 minutes.
10. Wash slides in distilled water for 1 minute.

Immunostaining with HRP-DAB Detection System

1. Apply peroxidase block (3% hydrogen peroxide) to the slides and incubate for 5 minutes.
2. Rinse slides in 1X TBST for 1 minute.
3. Apply Universal Protein Block (DAKO, Cat. #X0909) to the slides and incubate for 20 minutes.
4. Drain protein block from the slides.
5.

Apply primary antibody to the slides and incubate for 45 minutes.

6. Rinse slides in 1X TBST for 1 minute.
7. Apply LSAB2 System-HRP LINK solution (DAKO, Cat. #K0679) to the slides and incubate for 15 minutes.
8. Rinse slides in 1X TBST for 1 minute.
9. Apply LSAB2 System-HRP Streptavidin-HRP solution (DAKO, Cat. #K0679) to the slides and incubate for 10 minutes.
10. Rinse slides in 1X TBST for 1 minute.
11. Apply prepared DAB Substrate-Chromogen solution (DAKO, Cat. #K3468) to the slides and incubate for 5 minutes.
12. Rinse slides in 1X TBST for 1 minute.

Counterstaining with Hematoxylin

1. Stain slides with 65% Harris' Hematoxylin for 1 minute. Hematoxylin stains nucleic acids (nuclei) a deep blue-purple.

Dehydration and Coverslipping

This method should only be used if the chromogen substrate used is alcohol insoluble (e.g. Vector Red or DAB).

1. Wash slides in 2 changes of 80% alcohol for 1 minute each.
2. Wash slides in 2 changes of 95% alcohol for 1 minute each.
3. Wash slides in 3 changes of 100% alcohol for 1 minute each.
4. Wash slides in 3 changes of xylene for 1 minute each.
5. Apply coverslip with a drop of permanent mounting medium.