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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).
- Heat slides in a tissue-drying oven for 45 minutes at 60°C.
- Wash slides in 3 changes of xylene for 5 minutes each at room temperature.
- Wash slides in 3 changes of 100% alcohol for 3 minutes each at room temperature.
- Wash slides in 2 changes of 95% alcohol for 3 minutes each at room temperature.
- 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.
- 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.
- 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).

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