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Prestige Antibodies®
Powered by ATLAS ANTIBODIES

Immunohistochemistry Procedure

Product Description
The Prestige Antibodies® are subjected to a standardized test procedure using specially designed tissue microarray (TMA) slides.

Preparation Instructions
Deparaffinization
Paraffin sections of 4 µm thickness are baked overnight at 50 °C. Prior to immunostaining, deparaffinization and hydration are done in xylene and graded ethanol to distilled water. During hydration, a 5 minute blocking for endogeneous peroxidase is done with 0.3% (v/v) H_2O_2 in 95% ethanol.

Wash Buffer – working wash buffer contains 0.2% TWEEN® 20.

Procedure
Standard Antigen Retrieval Method
Heat Induced Epitope Retrieval (HIER) is performed by heating the TMA slides immersed in retrieval solution: 10 mM sodium citrate buffer, pH 6.0, with 1 mM EDTA, at 125 °C for 4 minutes in a pressure boiler. After boiling is completed, slides remain in the pressure boiler and are allowed to cool down to 90 °C. The total processing time is ~45 minutes.

Alternative Antigen Retrieval Methods
1. HIER performed with retrieval buffer, pH 9.
2. Enzymatic Antigen Retrieval - Enzymatic retrieval is performed by incubation of the TMA slides with Proteinase K for 10 minutes at room temperature (RT).

Standard primary antibody dilutions
- for antibody concentrations <0.06 mg/ml, 1:25
- for antibody concentrations >0.06 mg/ml, 1:75
- for antibody concentrations >0.1 mg/ml, 1:150
  Note: The specified working dilutions of the antibodies are to be considered as guidelines only. Optimal dilutions must be determined by the user.

Immunohistochemical staining – Performed with Lab Vision Autostainer™ 480. All incubations are performed at room temperature. Reagents are applied at a volume of 300 µl per TMA slide.

1. Rinse in wash buffer.
2. Incubation with Ultra V Block for 5 minutes.
3. Rinse 2 times in wash buffer.
4. Incubate with primary antibody for 30 minutes.
5. Rinse 3 times in wash buffer.
6. Incubate with peroxidase labeled polymer conjugated to a secondary antibody for 30 minutes.
7. Rinse 2 times in wash buffer.
8. Develop for 5 minutes using diaminobenzidine (DAB) as the substrate.
9. Rinse 2 times in wash buffer.
10. Develop for 5 minutes using DAB as the substrate.
11. Rinse 2 times in distilled water
    Note: Steps 12–17 are done in a histostaining instrument (Leica Autostainer XL).
12. Counterstain in Mayer's hematoxylin for 5 minutes.
13. Rinse 2 times in tap water.
14. Rinse in lithium carbonate water, diluted 1:5 from saturated solution, for 1 minute.
15. Rinse in tap water for 5 minutes.
16. Dehydrate in graded ethanol and Tissue Clear.
17. Coverslipping.

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