

## NBP2-75985 Protocol

### Immunohistochemistry protocol for MUC6 Antibody (NBP2-75985)

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Immunohistochemistry Protocol

#### Specimen Collection and Preparation for Analysis

Each tissue section should be fixed with 10% neutral buffered formalin, cut to the applicable thickness (4µm), and placed on a glass slide that is positively charged. The prepared slide may then be baked for a minimum of 30 minutes in a 53-65 degrees C oven (do not exceed 24 hours).

#### Recommended Staining Protocols

##### Manual Use:

1. Pretreatment: Perform heat-induced epitope retrieval (HIER) at pH 9 for 10 to 30 minutes.
2. Peroxide Block: Block in peroxidase blocking solution for 5 minutes at room temperature. (Not required if using Alkaline Phosphatase System.)
3. Primary Antibody: Apply antibody directly (Predilute) or dilute antibody at 1:100-1:200 (Concentrate) before applying. Incubate antibody for 10 to 30 minutes at room temperature.
4. Secondary Antibody: Incubate for 20 to 30 minutes at room temperature.
5. Substrate Development: Incubate DAB or Fast Red for 5 to 10 minutes at room temperature.
6. Counterstain: Counterstain with hematoxylin for 0.5 to 5 minutes, depending on the hematoxylin used. Rinse with distilled water and blueing solution for 30 seconds.
7. Dehydrate and apply coverslip.

##### Automated Staining System:

The stated primary antibody has been optimized and validated using the BOND-MAX fully automated IHC & ISH stainer manufactured by Leica Biosystems, applying IHC Protocol F. The following edits are recommended for the protocol:

- a) Marker Incubation Time: 30 minutes
- b) Heat-induced epitope retrieval (HIER) is recommended using Leica Bond ER Solution 2 for 30 minutes.
- c) Move Peroxide Block step to after Polymer and before Mixed DAB Refine. For all other automated IHC staining systems, refer to the corresponding user manual for specific instructions.