

## NBP3-11709 Protocol

### Immunohistochemistry protocol for MDM2/HDM2 Antibody (NBP3-11709)

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[https://www.novusbio.com/products/mdm2-hdm2-antibody-ihc620\\_nbp3-11709](https://www.novusbio.com/products/mdm2-hdm2-antibody-ihc620_nbp3-11709)  
Immunohistochemistry Protocol

#### Specimen Collection and Preparation for Analysis

Each tissue section should be fixed with 10% neutral buffered formalin, cut to the applicable thickness (4um), 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. Blocking: If HRP is used, block with peroxidase blocking solution for 10 to 15 minutes at room temperature. Use alkaline phosphatase blocking solution if using an AP system.
3. Primary Antibody: Apply antibody directly. Incubate antibody for 30 to 60 minutes at room temperature or overnight at 4C.
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 bluing solution for 30 seconds.
7. Dehydrate and apply coverslip.

For all automated IHC staining systems, refer to the corresponding user manual for specific instructions.