

NB100-105 Protocol

Immunohistochemistry-Paraffin Protocol for HIF-1 alpha Antibody (NB100-105)

Immunohistochemistry Protocol:

1. Prepare tissue with formalin fixation and by embedding it in paraffin wax.
 2. Make 4-mm sections and place on pre-cleaned and charged microscope slides.
 3. Heat in a tissue-drying oven for 45 minutes @ 60 degrees Celcius.
 4. Deparaffinize the tissues by wash drying the slides in 3 changes of xylene for 5 minutes each @ RT.
 5. Rehydrate the tissues by washing the slides in 3 changes of 100% alcohol for 3 minutes each @ RT.
 6. Wash the slides in 2 changes of 95% alcohol for 3 minutes each @ RT.
 7. Wash the slides in 1 change of 80% alcohol for 3 minutes @ RT.
 8. Rinse the slides in gentle running distilled water for 5 minutes @ RT.
 9. Perform antigen retrieval by steaming the slides in 0.01M sodium citrate buffer (pH 6.0) @ 99-100 degrees Celcius for 20 minutes.
 10. Remove the slides from the heat and let stand in buffer @ RT for 20 minutes.
 11. Rinse the slides in 1X TBS-T for 1 minute @ RT.
- **Do not allow the tissues to dry at any time during the staining procedure****
12. Begin the immunostaining by applying a universal protein block for 20 minutes @ RT.
 13. Drain protein block from the slides and apply the diluted primary antibody for 45 minutes @ RT.
 14. Rinse the slide in 1X TBS-T for 1 minute @ RT.
 15. Apply a biotinylated anti-rabbit IgG (H+L) secondary for 30 minutes @ RT.
 16. Rinse the slide in 1X TBS-T for 1 minute @ RT.
 17. Apply an alkaline phosphatase streptavidin for 30 minutes @ RT.
 18. Rinse the slide in 1X TBS-T for 1 minute @ RT.
 19. Apply an alkaline phosphatase chromogen substrate for 30 minutes @ RT.
 20. Rinse the slide in distilled water for 1 minute @ RT.
- **This method should only be used if the chromogen substrate is alcohol insoluble (ie: Vector Red, DAB)****
21. Dehydrate the tissue by washing the slides in 2 changes of 80% alcohol for 1 minute each @ RT.
 22. Wash the slides in 2 changes of 95% alcohol for 1 minute each @ RT.
 23. Wash the slides in 3 changes of 100% alcohol for 1 minute each @ RT.
 24. Wash the slides in 3 changes of xylene for 1 minute each @ RT.
 25. Apply cover slip.