

## NLS3305 Protocol

### Immunohistochemistry Protocol for CysLT2 Antibody (NLS3305)

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#### Immunohistochemistry

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 5 minutes each @ RT.
  5. Rehydrate the tissues by washing the slides in 3 changes of 100% alcohol 3 minutes each @ RT.
  6. Wash the slides in 2 changes of 95% alcohol 3 minutes each @ RT.
  7. Wash the slides in 1 change of 80% alcohol 3 minutes @ RT.
  8. Rinse the slides in gentle running distilled water 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 20 minutes @ RT.
  13. Drain protein block from the slides and apply the diluted primary antibody 45 minutes @ RT.
  14. Rinse the slide in 1X TBS-T 1 minute @ RT.
  15. Apply a biotinylated anti-rabbit IgG (H+L) secondary 30 minutes @ RT.
  16. Rinse the slide in 1X TBS-T 1 minute @ RT.
  17. Apply an alkaline phosphatase steptavidin 30 minutes @ RT.
  18. Rinse the slide in 1X TBS-T 1 minute @ RT.
  19. Apply an alkaline phosphatase chromagen substrate 30 minutes @ RT.
  20. Rinse the slide in distilled water 1 minute @ RT.
- \*\*This method should only be used if the chromagen substrate is alcohol insoluble (ie: Vector Red, DAB)\*\***
21. Dehydrate the tissue by washing the slides in 2 changes of 80% alcohol 1 minute each @ RT.
  22. Wash the slides in 2 changes of 95% alcohol 1 minute each @ RT.
  23. Wash the slides in 3 changes of 100% alcohol 1 minute each @ RT.
  24. Wash the slides in 3 changes of xylene 1 minute each @ RT.
  25. Apply cover slip.