Immunohistochemistry Protocol (Frozen)

1. Wash sections in 1X TBS two times for 5 minutes.

2.Incubate for 10min at Room temperature with 3%H_2O_2 .(30%H_2O_2 and dH2O volume ratio of 1:9)

3. Wash sections in 1X TBS two times for 5 minutes.

4. Block each section with 5% Normal Serum(derived from the same species in which the secondary antibody was raised.) for 1 hour at room temperature.

5. Remove blocking solution and add 100–400 µl of primary antibody diluted in 1X TBS/0.3% Triton X-100/5% Normal Goat Serum to each section.

6. Incubate overnight at 4°C.

7. Return to room temperature.

8. Remove antibody solution and Wash sections in 1X TBST 1 times for 5 min, then TBS 3 times for 5min per time.

9. Incubate in humidified box for 30 min at Room temperature with secondary antibody. 10. Wash sections in 1X TBST 1 times for 5 min, then TBS 3 times for 5min per time.

11. Prepare an appropriate volume of substrate solution prior to use by mixing one drop of Liquid DAB plus chromogen immediately with 1 ml of substrate buffer. Apply the substrate carefully and incubate for 5-10 minutes till a brown color develops.

12. Immerse slides in dH2O.

13. To stain nuclei, immerse slides in a bath of hematoxylin for 6-10S.

14. Rinse slides gently with distilled water for 10min.

15. Transfer slides into a 1% HCl, 99% ethanol solution for 10 seconds; transfer to distilled

water immediately.

16. Wash sections in dH2O two times for 5 minutes each.

17. Dehydrate sections: Incubate sections in 95% ethanol two times for 10 seconds each. Repeat the incubation process in 100% ethanol. Repeat again in xylene.

18. Wipe off excess xylenes. Immediately add 1–2 drops of Neutral resin,cover with a glass coverslip and observe by light microscopy.