High Temperature Antigen Unmasking Technique Using Na-citrate Buffer or IHC with Paraffin Sections

1. Cut and mount sections on slides coated with Vectabond (cat.# SP1800, Vector Laboratories) or Apes (3-aminopropyltriethoxysilane, cat# A3648, Sigma Immunochemicals).

2. Deparaffinize sections and rehydrate with Distilled water.

3. Bring 1.6 l of 0.01M sodium citrate (pH 6) to a boil in a Prestige stainless steel pressure cooker, using a hot plate; cover but do not lock lid.

4. Position slides into metal staining racks and lower into cooker ensuring slides are well immersed in buffer. Lock lid. The small valve will rise.

5. When pressure indicator valve (large one) has risen after about 4 min, incubate sections for 1 min.

6. Remove cooker from heat and run under cold water with lid on. When the small valve sinks, open lid and remove slides and place immediately into dist. Water. Don't open lid until the small valve sinks.

7. Wash sections in TBS (pH7.6) for 1 x 5 min).

8. Place sections in 1.5% hydrogen peroxide/methanol for 10 min.

9. Wash sections in dist. Water for 2 x 5 min; then wash sections in TBS buffer for 2 x 5 min.

10. Place sections in normal serum for 20 min.

11. Cover sections with the primary AB (conditions should be optimized for each system or lab).

12. Wash in TBS buffer for 2x5 min.

13. Incubate sections with secondary AB for 30 min.

14. Was in TBS buffer for 2 x 5 min.

15. Incubate slides in ABComplex for 30 min.

16. Wash in TBS buffer for 2 x 5 min.

17. Incubate slides in DAB .

18. Wash in water for 2 x 5 min.

19. Counterstain with hematoxylin (as needed), dehydrate, coverslip mount and view.