

High Temperature Antigen Unmasking Technique using EDTA for Immunohistochemical Demonstration on Paraffin Sections.

1. Cut and mount sections on slides coated with Vectabond* or Apes**.
2. Deparaffinize sections and rehydrate to distilled water.
3. Bring 1600ml 1mM EDTA (pH 8.0) to the 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 pressure cooker ensuring slides are well immersed in 1mM EDTA. Lock lid. The small valve will rise.
5. When the pressure indicator valve (the large one) has risen after about 4 minutes, incubate sections for 1 minute.
6. Remove pressure cooker from heat source and run under cold water with lid on. When the small valve sinks open lid and remove slides and place immediately into distilled water. **DO NOT OPEN LID UNTIL THE SMALL VALVE SINKS.**
7. Wash sections in TBS buffer (pH 7.6) for 1 x 5 minutes.
8. Place sections in 1.5% hydrogen peroxide/methanol for 10 minutes.
9. Wash sections in distilled water for 2 x 5 minutes, then wash sections in TBS buffer for 2 x 5 minutes.
10. Place sections in normal serum for 20 minutes.
11. Cover sections with primary antibody. (The optimal dilution of the antibody, incubation time and incubation temperature should be determined by the individual laboratory).
12. Wash in TBS buffer for 2 x 5 minutes.
13. Incubate sections in secondary antibody for 30 minutes.
14. Wash in TBS buffer for 2 x 5 minutes.
15. Incubate slides in ABCComplex for 30 minutes.
16. Wash in TBS buffer for 2 x 5 minutes.
17. Incubate slides in DAB.
18. Wash in water for 2 x 5 minutes.
19. Counterstain with haematoxylin (if required), dehydrate, coverslip and mount.

*Vectabond: (Catalogue No SP1800) Vector Laboratories

**APES (3-aminopropyltriethoxysilane) (Catalogue No A3648) Sigma Immunochemicals

To avoid sections becoming detached, sections should be mounted on "Vectabond" or "APES" covered slides, then dried at 37°C overnight followed by drying at 56°C for 60 minutes.