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## HIGH TEMPERATURE ANTIGEN UNMASKING TECHNIQUE FOR IMMUNOHISTOCHEMICAL DEMONSTRATION ON PARAFFIN SECTIONS

1. Cut and mount sections on slides coated with VECTABOND™ Reagent tissue adhesive.
2. Deparaffinize sections and rehydrate to distilled water.
3. Place sections in 0.5% hydrogen peroxide/methanol for 10 minutes (or use other appropriate endogenous peroxidase blocking procedure). Wash sections in tap water.
4. Heat 1500ml of diluted antigen unmasking solution (unless otherwise indicated overleaf) until boiling in a stainless steel pressure cooker. Cover but do not lock lid.
5. Position slides into metal staining racks (do not place slides close together as uneven staining may occur) and lower into pressure cooker ensuring slides are completely immersed in unmasking solution. Lock lid.
6. When the pressure cooker reaches operating temperature and pressure (after about 5 minutes) start a timer for 1 minute (unless otherwise indicated on the data sheet).
7. When the timer rings, remove pressure cooker from heat source and run under cold water with lid on. DO NOT OPEN LID UNTIL THE INDICATORS SHOW THAT PRESSURE HAS BEEN RELEASED. Open lid, remove slides and place immediately into a bath of tap water. (Circle sections with the hydrophobic barrier ImmEdge™ Pen, if required.)
8. Wash sections in TBS\* buffer (pH 7.4) for 1 x 5 minutes.
9. Place sections in diluted normal serum for 10 minutes.
10. Incubate sections with primary antibody.
11. Wash in TBS buffer for 2 x 5 minutes.
12. Incubate sections in an appropriate biotinylated secondary antibody.
13. Wash in TBS buffer for 2 x 5 minutes.
14. Incubate slides in VECTASTAIN® Elite® ABC reagent (or VECTASTAIN® Universal Quick Kit).
15. Wash in TBS buffer for 2 x 5 minutes.
16. Incubate slides in DAB or other suitable peroxidase substrate.
17. Wash thoroughly in running tap water.
18. Counterstain with hematoxylin (if required), dehydrate, clear and mount.

### SOLUTIONS

1. Vector® Antigen Unmasking Solution, low pH, (H-3300).
2. 1mM EDTA (pH 8.0): Add 0.37g of EDTA to 1 liter of distilled water. Adjust pH to 8.0 using 1.0M NaOH.
3. Vector® Antigen Unmasking Solution, high pH, (H-3301).

\* In most applications, 10mM phosphate, 0.15 M NaCl, pH 7.4 (PBS) can be used instead of 50 mM Tris, 0.15 M NaCl, pH 7.4 (TBS).

### SAFETY NOTE

To ensure the correct and safe use of your pressure cooker, PLEASE READ THE MANUFACTURER'S INSTRUCTIONS.