Immunohistochemistry:
Immunoperoxydase staining for frozen tissue sections

1. Leave frozen tissue sections warm at room temperature for 1 to 2 hours.
2. Fix the sections in cold acetone for 10 min.
3. Wash twice in PBS.
4. If necessary, block endogenous peroxydase by incubating in 0.1% H_2O_2 in 70% methanol for 10-30 minutes.
5. Wash once in PBS.
6. Incubate sections for 1 hour in 1.5% normal blocking serum in PBS, derived from the same species in which secondary has been raised. Remove blocking serum from slides.
7. Incubate with appropriately diluted primary antibody for at least 1 hour at room temperature or overnight at 4°C. Wash twice in PBS.
8. Incubate the sections with the peroxydase conjugated secondary antibody at recommended dilution. Incubate for 30-60 minutes at room temperature. Wash twice in PBS.
9. Stain with the appropriate substrate DAB solution or AEC solution for 10 minutes.
10. Wash with demineralised water.
11. Briefly counterstain with haematoxylin for 1-10 minutes.
12. Wash gently in running water until blue colour is clearly visible.
13. Dehydrate by increasing solution of ethanol and xylene solvent, mount with medium and examine by light microscopy.

DIACLONE SAS - 6 Rue Docteur Jean-François-Xavier Girod - BP 1985- 25020 BESANCON Cedex - France
Tel: +33(0)3.81.41.38.38 - Fax: +33(0)3.81.41.36.36
info@diaclone.com - www.diaclone.com