# Immunohistochemical staining procedure

We employ routine immunnostaining procedures for the demonstration of nuclear receptor localization in tissues without using specific secondary antibodies or buffer kits. Following is the procedure to stain formalin-fixed and paraffin-embedded specimens.

1. Four micron-thick sections should be used.
2. Dewax in xylol, 3 x 5 min
3. Place the slides into glass slide chamber and fill them with the processing buffer (citric acid buffer, pH6.0, preferably pH6.5-7.0)
4. Antigen retrieval by autoclave (121℃, 15min).
5. Take out the glass slides in chamber, wait for 40 min, and rinse the slides 3 times with distilled water or PBS by emptying and refilling the chambers.
6. Block endogenous peroxidase activity with freshly made 0.3% $H_20_2$ in methanol, 20 min.
7. Three changes of PBS for five minutes.
8. Incubate with monoclonal antibody, 4℃, overnight.
9. Wash with PBS, 3 x 5 min.
10. Incubate with second antibody,MAX-PO(MULTI), 60-120 min. at room temperature.
11. Three changes of PBS for five minutes.
12. Stain with diaminobenzidin (DAB) solution, 10 min. at room temperature. 0.01% DAB in 0.5 M Tris/HCI (pH 7.4) solution should be filtrated. $H_20_2$ must be added to a final concentration of 0.01%.
13. Wash with running tap water, 3 min.
14. Counterstain with Mayer's hematoxylin, 0.5 min.
15. Wash with running tap water.
16. Dehydrate with increasing solutions of ethanol: 50%, 70%, 96%, absolute, 3 min. each.
17. Clear with xylol, 3 x 3 min.
18. Mount with mounting medium.

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