Single Biotinylated Antibody or PNA Staining

All washes and dilutions are with PBS + 5% BSA + 0.1% Tween 20.
All incubations and washes are at room temperature.

1. Remove slides from the freezer and allow to come to room temperature.
2. Make a circle around the tissue section with PAP pen (Research Products, Cat. #195500). Allow to dry for 15-30 minutes.
3. Incubate slides in PBS (without azide) for 30 minutes.
4. Incubate slides in PBS + 5% BSA + 0.1% Tween 20 for 30 minutes.
5. Dry slide outside of PAP pen circle.
6. Apply 100 µl of antibody or PNA dilution to tissue section and incubate in a humidified chamber for 1 hour.
7. Wash slides 3 X 10 minutes on a shaker.
8. Wipe off slide outside of ring and incubate with 100 µl of Streptavidin-AP (1:400 dilution) for 45 minutes in the humidified chamber.
9. Wash slides 3 X 10 minutes on a shaker.
10. Apply 100 µl of NBT/BCIP substrate to the tissue section and incubate in the humified chamber in the dark. Visualize under light microscope.
11. Mount with Crystal Mount (Biomeda, Fisher Cat #BM-M02).

Substrate:

0.1M Tris-HCl, pH 9.5	1 ml of 1M Tris-HCl, pH 9.5
0.1M NaCl	0.2 ml of 5M NaCl
50 mM $MgCl_2$	0.5 ml of 1M $MgCl_2$
0.5% Levamisole	
Total	10 ml

To 10 ml of substrate add 44 µl NBT and 33 µl of BCIP (Gibco/BRL, Cat# 8280SA).

Cover tube with foil and use immediately.