

## Protocol for Immuno-Histochemistry on Sections

1. Clear Slides  
Xylene (*2x 2 min*)
2. Rehydrate  
100% EtOH, 95% EtOH, 70% EtOH, 2x dH<sub>2</sub>O (*2 min each*)
3. PBS Wash (*2x 3 min*)
4. Antigen Retrieval  
Microwave to boil  
Steam (*30 min*)  
Cool uncovered on bench (*30+ min*)  
\*\*DO NOT USE GLASS
5. PBS Wash (*2x 3 min*)
6. Block in 5% normal goat serum in PBS (*1 hour*)
7. 1° Ab in 5% goat serum (*2 hr*)
8. Wash in PBS-T (*4x 15-20 min*)  
0.1 % Tween
9. 2° Ab in 5% goat serum (*45 min*)  
\*\*IN DARK
10. Wash in PBS-T (*4x 15-20 min*)  
0.1 % Tween  
Hoechst (1:1000) in wash 3  
\*\*IN DARK
11. Rinse in dH<sub>2</sub>O and cover with anti-fade