

**Immunohistochemistry Protocol for use on Paraffin Sections,**

Date: _____ **Assay:** _____ **Samples/# of slides:** _____
Washing Buffer: _____ **Diluting Buffer:** 1%BovineSerumAlbumin or equivalent in Wash Buffer

1. Air Dry Slides for 3 days and proceed to fume hood to start **Deparaffinization** in the fume hood:
___ 3x Xylene for 10 min each (**30 minutes total**) and then can do the rest on the bench
___ **Rehydration on bench** ___ 2x 100% EtOH for 5 min each (**10 minutes total**)
___ 2x 95% EtOH for 5 min each (**10 minutes total**)
___ 2x 70% EtOH for 5 min each (**10 minutes total**), followed by immersion 3x in Wash Buffer

2. Blocking Steps:

- a. Block Endogenous Peroxidase (if using peroxidase substrate)
___ 0.3% H₂O₂ in Wash Buffer for **30 minutes**, followed by 3x Wash Buffer
 - b. Block Hydrophobic Interactions: Apply 1%BovineSerumAlbumin or equivalent in Wash Buffer
 - c. Block Endogenous Biotin (if using biotinylated antibodies)
___ Apply 0.1% Avidin from blocking kit for **15 minutes**, followed by 3x Wash Buffer
___ Apply 0.01% Biotin from blocking kit for **15 minutes**, followed by 3x Wash Buffer
3. **If needed: Antigen Retrieval:** ___
 - a. Heat-Induced Epitope Retrieval (HIER) **CHOOSE pH of Retrieval solution**
___ *place slides in complete rack, use dummy slides as necessary*
___ Heat slides in a Citrate Buffer w/ water bath for **5 minutes**
___ Check slides and then repeat for **5 minutes**
___ Allow cool down at room temp for **exactly 20 minutes**

OR

- b. Enzyme-Induced Epitope Retrieval
___ Proteinase K for **7 minutes** followed by 3x Wash Buffer

4. Primary Application

#	Primary Reagents	Catalog Number	Dilutions	Incubation Time
1.			:	
2.			:	
3.			:	
4.			:	
5.			:	

___ 3x Wash Buffer

5. Secondary Application

#	Secondary Reagents	Slides Applied To/Groups Applied	Dilutions	Incubation Time
1.			:	

followed by ___ 3x Wash Buffer

6. Tertiary Application

#	Tertiary Reagents	Slides Applied To/Groups Applied	Dilutions	Incubation Time
1.			:	

followed by ___ 3x Wash Buffer

7. Substrate Application ___

- a. Reagent: _____ apply for _____ **minutes** followed by 3x Wash Buffer
8. **Counterstain:** _____ for _____ **minutes** followed by 3x Wash Buffer
 9. Air Dry Slides before applying Coverslips using proper medium (_____)
 10. Use correct Microscope for digital photographs and record results