

## **IHC Protocol**

### **Tissue Sectioning**

- Tissue is fixed in formalin.
- Tissue should be cut into 4-5  $\mu\text{m}$  sections on clean, charged microscope slides and then heated in a tissue-drying oven for 45 minutes at 60°C

### **Deparaffinization:** At Room Temperature

- 5 minutes each: wash slides in 2 changes of xylene

### **Rehydration:** At Room Temperature

- 5 minutes each: wash slides in 2 changes of 100% alcohol
- 5 minutes each: wash slides in 2 changes of 95% alcohol
- 3 minutes each: wash slides in 1 change of 75% alcohol
- 5 minutes: gently rinse slides using PBS

### **Antigen Retrieval:**

- Heat antigen retrieval reagent (antigen unmasking solution, H-3300, Vector Laboratories) at 95°C for 9 minutes
- Keep all slides in the reagent and heat at 95°C for 3 minutes in 3 changes
- Let slides cool in reagent at room temp for 20 minutes
- Rinse 3 x with PBS (1x) at room temp. for 5 mins each

### **Immunostaining:** Tissues should not dry at any time during the procedure!

- ❖ Block endogenous antigen and peroxidase antibody:
  - Incubate with 1%  $\text{H}_2\text{O}_2$  (H325-100, Fisher Scientific) for 30 mins at room temp.
  - Rinse 3 X with PBS (1x) at room temp. for 5 mins each
  - Incubate with 3% bovine serum albumin (BSA) at room temp for 1hr
- ❖ Incubate with primary antibody and secondary antibody:
  - Dilute primary AR antibody (ab133273, 1:200, abcam) with 3% BSA in the ratio of (1:200)
  - Incubate in a humidified chamber with primary antibody at room temp for 1hr
  - Rinse 3 X with PBS (1x) at room temp. for 5 mins each
  - Incubate with secondary antibody (SignalStain Boost IHC Detection Reagent, # 8114, Cell Signaling) in a humidified chamber for 30 minutes at room temp.
  - Rinse 3 X with PBS (1x) at room temp. for 5 mins each
- ❖ Apply DAB chromogen substrate (#8059, SignalStain DAB substrate Kit, Cell Signaling):
  - Incubate with the working solution at room temp. until staining develops. 2 mins generally provides an acceptable staining intensity of AR staining on human BPH tissues.

### **Dehydration:** At Room Temperature

- 1 minute each: Wash slides in 2 changes of 75% alcohol
- 1 minute each: Wash slides in 2 changes of 95% alcohol

- 1 minute each: Wash slides in 2 changes of 100% alcohol
- 1 minute each: Wash slides in 2 changes of xylene
- Apply coverslip.