

## IHC Protocol

### 1. Deparaffinization

- a) Place slides in Xylene bath #1 for 3 minutes
- b) Place slides in Xylene bath #2 for 3 minutes

### 2. Rehydration

- a) Place slides in 100% ethanol bath #1 for 3 minutes
- b) Place slides in 100% ethanol bath #2 for 3 minutes
- c) Place slides in 95% ethanol bath for 3 minutes
- d) Place slides in 70% ethanol bath for 3 minutes

### 3. Heat-induced epitope retrieval

- a) Wash slides in reagent water for 3 minutes
- b) Place slides in antigen retrieval buffer (Tris-EDTA, pH 9.0)
- c) Heat slides at 95 degree for 15-20 minutes. Allow slides cool down

### 4. Endogenous peroxidase

- a) Wash slides with reagent water for 3 minutes
- b) Incubate slides with 3% Hydrogen Peroxide for 10 minute

### 5. Staining

- a) Wash slides with 1X PBS for 3 minutes, 3 times
- b) Block slides with 5% goat serum/PBS for 1 hr
- c) Apply approximately 150  $\mu$ L of primary antibody (Rabbit anti-BRAF V600E, Clone RM8, 5 $\mu$ g/ml, diluted in 0.5% BSA/PBS) to each tissue section and incubate for 30 minutes at RT in a covered slide tray
- d) Wash slides with 1X PBS for 3 minutes, 3 times
- e) Apply approximately 150  $\mu$ L of Goat anti-rabbit HRP-Polymer (ready to use) and leave on for 15 minutes at RT in covered slide tray
- f) Wash slides with 1X PBS for 3 minutes, 3 times
- g) Apply approximately 100  $\mu$ L (~2 drops) of substrate-chromogen solution to tissue.
- h) Rinse slides with water
- i) Apply Hematoxylin to tissue sections
- j) Rinse with water

### 6 Mounting slides

- a) Place slides in 100% ethanol bath #1 for 3 minutes
- b) Place slides in 100% ethanol bath #2 for 3 minutes
- c) Place slides in Xylene bath #1 for 3 minutes
- d) Place slides in Xylene bath #2 for 3 minutes
- e) Applying Cover Slip to Stained Tissue Slides