IHC Protocol BRAF V600E Antibody Clone RM8

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 µL of primary antibody (Rabbit anti-BRAF V600E, Clone RM8, 5µg/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 µL 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 µL (~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