

## NBP2-36429 Protocol

### Immunohistochemistry-Paraffin Protocol for VEGF Receptor 2 Antibody (NBP2-36429)

1. Deparaffinize the tissue sections by immersing the slides in Xylene with two changes for 10 min each. Sections should not get dried at any stage from this point.
2. Rehydrate the tissue sections by immersing the slides in decreasing grades of ethanol as follows:
  - a. Immerse in 100% ethanol with 2 changes for 5 minutes each
  - b. Immerse in 95% ethanol with 2 changes for 5 minutes each
  - c. Immerse in 90% ethanol for 5 minutes
  - d. Immerse in 70% ethanol for 5 minutes
  - e. Immerse in 50% ethanol for 5 minutes
  - f. Immerse in distilled water for 5 minutes
3. Antigen Retrieval (Microwave Method):
  - a. Immerse the slides in a microwave compatible tray containing 10 mM Sodium Citrate buffer (pH 6.0) with 0.05% Tween 20.
  - b. Boil the slides and maintain the sub-boiling temperature for 5 minutes in the microwave. Thereafter, take out the tray very carefully and cool it at room temperature (RT) for about 30 minutes.
  - c. Wash the slides 3 times, 3 minutes each by immersing them in TBST (Tris Buffered Saline having 0.05% Tween 20).
4. Quenching of Endogenous Peroxidase:
  - a. Incubate the slides in 3% hydrogen peroxide prepared in methanol for 15 minutes (at RT, in dark conditions).
  - b. Wash the slides in TBST 3 times, 3 minutes each.
5. Protein Blocking:
  - a. Incubate the sections with background sniper solution at RT for 15 minutes (Biocare Medicals, USA).
  - b. Wash the sections 3 times, 3 min each by immersing the slides in TBST.
6. Primary Antibody:
  - a. Dilute the primary antibody at 5ug/ml concentration using PBS as a diluent.
  - b. Incubate the sections with diluted primary antibody for 90 minutes at RT in a humidified chamber.
  - c. Thereafter, wash the slides 4 times, 5 minutes each with TBST.
7. Probe (Secondary Reagent):
  - a. Incubate with MACH 1 Mouse probe for 15 minutes at RT.
  - b. Incubate for 30 min at room temperature with HRP-Polymer (Biocare Medical, USA).
  - c. Wash the slides with TBST 4 times, 5 minutes each
8. Chromogen:
  - a. Mix 32ul of DAB Chromogen with 1 ml of DAB substrate buffer (Biocare Medical, USA).
  - b. Apply 200ul DAB mixture/section and incubate at RT in dark conditions (few seconds - 5 minutes).
  - c. As soon as an appropriate color develops, rinse the slides with deionized water (2-3 brief rinses).
9. Counter stain:
  - a. Counter stain with Hematoxylin for 30 seconds (Vector Labs, USA).
  - b. Wash in deionized water for 1-2 minutes to clear the extra stain.
  - c. Incubate the slides in bluing solution or Scott's water twice for 2 minutes each time.
10. Dehydrate the sections in increasing grades of alcohols:
  - a. 50% alcohol for 1 minute
  - b. 70% for 1 minute
  - c. 90% for 1 minute
  - d. 95% for 1 minute
  - e. 100% for 1 minute
  - f. Xylene with 2 changes for 2 minutes each
11. Mount with DPX mount and cover-slip glass (Fisher Scientific, USA), carefully not allowing any air bubbles to enter.

NOTE:- This protocol is provided as a reference tool only. Depending upon the type of tissues /tissue processing and reagents employed, the end user will need to optimize the final conditions for achieving an expected staining.