

## NB110-97871 Protocol

### Immunohistochemistry protocol for CD11c Antibody (NB110-97871)

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1. Dewax and Rehydration: Place the slides in a rack and sequentially pass the rack through following solutions: HistoClear or Xylene twice, 10 minutes each; and then a graded series of 100%, 90%, 70%, and 50% ethanol, 2 minutes each.
2. Wash the slides with tap water for 5 minutes and once with DI water.
3. Antigen Retrieval: treat the slides with 20ug/ml proteinase K in PBS for 15-25 minutes at room temperature.
4. Wash the slides with tap water for 5 minutes and quench the endogenous peroxidase with 3% H<sub>2</sub>O<sub>2</sub> in DI water for 30 minutes
5. Wash the slides with tap water for 5mins and soaking in PBS-Tween20 (0.1%)
6. Block the slides with blocking buffer (25% bovine serum in PBS-Tween20) for at least 10minutes
7. Then incubate the specimen with primary antibody (NB110-97871, dissolved with 200ul PBS) at 1:50-200 dilutions overnight at 4 degree (or room temperature if the signal is too weak).
8. Wash the slides with rotation in PBS-Tween20 for 3 times, 5 minutes each.
9. Incubate the specimen with rabbit-anti Hamster secondary antibody for 30minutes at room temperature.
10. Wash the slides with rotation in PBS-Tween 20 for 3 times, 5 minutes each.
12. Incubate the specimen with Polymer-HRP labeled anti rabbit (Dako: K4010 Envision Kit) for 30 minutes at room temperature.
13. Wash the slides with rotation in PBS-Tween20 for 3 times, 5 minutes each.
14. Perform DAB Color development (reagents available in Dako K4010).
15. Wash with tap water and count stain the nuclear with hematoxylin.

Note:

- 1) 1) If you use buffered Formalin fixatives, we recommend that Fixation should be for no longer than 1 day (4 degree preferred).
- 2) This protocol is for reference only and the final condition should be optimized by the end user.