

**Immunohistochemistry Protocol for CD4** (Cat#103-M105)

1. **Dewax and Rehydration**: Place the slides in a rack and sequentially pass the rack through following solutions: HistoClear or Xylene twice, 10 min each; and then a graded series of 100%, 90%, 70%, and 50% EtOH, 2 min each.
2. Wash the slides with tap water for 5 min and once with DI water.
3. **Antigen Retrieval**: treat the slides with 20µg/ml Proteinase K in PBS for 15-25 min at RT.
4. Wash the slides with tap water for 5 min and quench the endogenous peroxidase with 3% H<sub>2</sub>O<sub>2</sub> in DI water for 30 min.
5. Wash the slides with tap water for 5 min and soaking in PBS-Tween20 (0.1%).
6. Block slides with blocking buffer (25% bovine serum in PBS-Tween20) for at least 10 min.
7. Then incubate the specimen with primary antibody (dissolved with 200µl PBS) at 1:25-100 dilutions overnight at 4 degree (or RT if the signal is weak).
8. Wash slides with rotation in PBS-Tween20 for 3 times, 5 min each.
9. Incubate the specimen with rabbit-anti rat secondary antibody (Vector Laboratory, BA-4001, 0.5mg/ml: 1:50-100) for 30 min at RT.
10. Wash the slides with rotation in PBS-Tween 20 for 3 times, 5 minutes each.
11. Incubate the specimen with Polymer-HRP labeled anti rabbit (Dako: K4010 **Envision Kit**) for 30 min at RT.
12. Wash the slides with rotation in PBS-Tween20 for 3 times, 5 min each.
13. Perform DAB Color development (reagents available in Dako K4010).
14. Wash with tap water and counter stain the nuclear with hematoxylin.

**Note:**

- 1) **We recommend that Formalin fixation should not be done no longer than 3 days (4 degree is preferred).**
- 2) **This protocol is for reference only and the final condition should be optimized by the end user.**