Immunohistochemistry Protocol for CD8a (Cat#103-M106)

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_2O_2 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 1:25-50 dilutions overnight at 4 degree (or RT if the signal is week).

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 Laborotary, 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 count 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.