

The protocols of immunohistochemistry Staining

(VECTASTAIN ABC Systems [<http://vectorlabs.com>] is suggested use in our TMAs)

1. Deparaffinize and dry array slide as referred to in protocol of deparaffinization.

2. Rinse array slide twice with PBS for 5 min each.

3. (Optional) The endogenous peroxidase activity is blocked at room temperature by a 5~10 min incubation in the final developmental 3% H₂O₂ in distilled water or PBS (pH 7.4).

4. Rinse array slide in PBS for 5 min.

5. Antigen retrieval.

6. Rinse array slide in PBS for 5 min.

7. Apply the blocking antibody (normal goat serum), incubate for 20 min at room temperature, and throw off residual fluid (don't wash.).

8. Apply the primary antibody 60 min. at RT or 4°C.

9. Rinse array slide twice for 5 min each.

10. Incubate array slide with a biotin-conjugated secondary antibody at 20~37°C for 20 min.

11. Rinse array slide twice for 5 min each.

12. Incubate array slide with SABC reagent at 37°C for 20 min.

13. Rinse array slide 4 times for 5 min each.

14. Proceed with chromogen of final developmental DAB or use DAB Kit (Control the degree of staining with regular microscopy).

15. Wash array slide in distilled water.

16. Stain and differentiate array slide in hematoxylin.

17. Dehydration and transparency of array slide.

18. Mount array slides.