

## NB110-90593 Protocol

### Immunohistochemistry Protocol for Ki67 Antibody (NB110-90593)

#### Immunohistochemistry

1. Slice fresh tissues in 3um sections
2. Formaldehyde fixation 50C for 1 hour
3. Dehydrate under 80%, 90%, 90%, 95%, 95%, 100% and 100% ethanol successively under 50C for 1 hour of each concentration
4. Xylene I 40C for 1 hour
5. Xylene II 40C for 1 hour
6. Paraffin I embedding 65C for 1 hour
7. Paraffin II embedding 65C for 1 hour
8. Paraffin III embedding 65C for 1 hour
9. Deparaffinize and rehydrate sections as standard
10. Antigen retrieval using 10mM sodium citrate microwave for 20 minutes
11. Wash and PBS wash as standard
12. Add 100ul primary antibody diluted to 1:3200 separately and incubate at 4C overnight
13. Wash in PBS buffer for 3 times 5 minutes
14. Remove excess PBS buffer and incubate sections with biotinylated secondary antibody, incubate for 30 minutes at 30C
15. Wash in PBS buffer for 3 times 5 minutes
16. Remove excess PBS buffer and incubate sections with ABC at 30C for 30 minutes
17. Wash in PBS buffer for 3 times 5 minutes
18. Develop with DAB
19. Rinse slides in gently running tap water for 10 minutes.