

NB100-304 Protocol

Immunohistochemistry-Paraffin Protocol for 53BP1 Antibody (NB100-304)

Materials

- 1) 1 Phosphate buffered saline (pH 7.6): NaCl 137mmol/L, KCl 2.7mmol/L, Na₂HPO₄ 4.3mmol/L, KH₂PO₄ 1.4 mmol/L
- 2) Citrate buffer, 0.01 M, pH6.0, Sodium Citrate 3g, Citric acid 0.4g
- 3) 3% Hydrogen peroxide
- 4) Primary antibody
- 5) Blocking serum (normal serum)
- 6) Biotinylated secondary antibody
- 7) DAB staining kit

Methods

1. Dewax and hydration of slides using xylene and EtOH:

Dry slides for 20 min in a 60 C oven

Add Xylene, 2 x 10 min

100%, 95%, 80%, and 70% EtOH, 5 min each EtOH concentration

Rinse in PBS, 5'

- 2 Antigen retrieval method (only for paraffin slides)

- 1a. High-pressure antigen retrieval procedure (recommended method)

Place slides in a glass slide holder (ensure that the slide holder is completely filled with slides, slides without sections if necessary, to ensure even heating. The entire slide holder is immersed in 1000 ml of Citrate buffer (0.01M, pH6.0) within a pressure cooker

Once steam is produced, and ONLY when steam is visible, from the pressure cooker (usually 15-20 min), the required high-pressure will have been reached, and slides will be incubated for 2 min.

Turn off heat, and allow buffer and slides to cool to room temperature

Slides are then rinsed in PBS for 5 minutes

2. Add 3% hydrogen peroxide solution, 10'at RT, then PBS, 3X5'

3. Normal blocking serum, 20'at RT

4. Incubate with Primary Ab, 4C overnight or 1.5 hours at 37C

5. Rinse with PBS, 3 X 5' each rinse

6. Add Biotin-conjugated second antibody, 10'at RT

7. Rinse with PBS, 3 X 5' each rinse

8. Add Streptavidin-Peroxidase, 10'at RT

9. Rinse with PBS, 3 X 5' each rinse

10. Staining with DAB solution, 2-5'under microscope

11. Stop the reaction by washing in tap water

12. Counterstain in Haematoxylin for 3-5 minutes

13. 75%, 80%, 95% and 100% ethanol, 5x2', xylene 2 x 10'