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# p53 immunohistochemistry protocol

PLOS One

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- 1 Deparaffinize and rehydrate sections:**  
Incubate 3 times in xylene for 5 minutes each.  
Incubate 3 times in 100% Ethanol for 5 minutes each.  
Hydrate by placing in 90%, 80% ethanol for 5 minutes each.  
Immerse slides in tap water for 5 minutes.
- 2 Sodium Citrate Antigen Retrieval:**
  - a. Place rack in Sodium Citrate buffer (10 mM, pH 6.0) in a glass beaker.
  - b. Autoclave for 10 minutes at 120 °C.
  - c. Immerse glass beaker in tap water to cool.
  - d. Immerse glass slides in tap water for 5 minutes.
- 3 Block endogenous peroxidases:**
  - a. Soak slides in 3% H<sub>2</sub>O<sub>2</sub> for 5 minutes at room temperature.
  - b. Wash once with PBS.
- 4 Blocking**  
Shake and wipe off excess PBS. Circle all sections with a DAKO pen. Add blocking buffer (10% normal goat serum) to each section immediately, so that the sections don't dry out. Don't touch sections with tip. Incubate for 20 minutes at room temperature in a humidified chamber. Wash in PBS for 5 minutes.
- 5 Primary antibody**  
Dilute primary antibody (anti-p53) in antibody dilution buffer (1:800).  
Add 50-75 µl per section and incubate overnight at 4 °C in a humidified chamber.
- 6 Drain primary antibody off section. Wash slides 3 times in PBS for 5 minutes.**
- 7 Dilute biotinylated secondary antibody. Add 50-75 µl per section and incubate more than 30 minutes at room temperature in a humid chamber.**
- 8 Drain secondary antibody and wash slides 3 times for 5 minutes each in PBS.**
- 9 Peroxidase visualization:**  
Mix 1 drop Vectastain "A" and 1 drop Vectastain "B" in 5 ml PBS (for 20 slides) as per kit instructions at the beginning of the secondary antibody incubation (30-60 minutes before use).  
Incubate 10-20 minutes in a humidified chamber. Rinse with buffer as before.
- 10 Incubate in fresh DAB solution.**  
(30 mg DAB + 30 µl 30% H<sub>2</sub>O<sub>2</sub> in 150 ml 0.05 M Tris pH 7.6)
- 11 Stop the reaction by washing in PBS.**

**12 Counterstain with hematoxylin.**

**13 Dehydrate sections:**

Incubate once in 70% Ethanol for 5 minutes.

Incubate 3 times in 100% Ethanol for 5 minutes each.

Incubate 3 times in xylene for 5 minutes each.

**14 Coverslip slides using Permount (xylene based).**