p53 immunohistochemistry protocol

PLOS One

Ratha-Korn Vilaichone¹, Natsuda Aumpan²

¹Gastroenterology Unit, Department of Medicine, Faculty of Medicine, Thammasat University Hospital, Department of Medicine, Chulabhorn International College of Medicine (CICM) at Thammasat University, Pathumthani, Digestive Diseases Research Center (DRC), Thammasat University Hospital, Pathumthani, Thailand;

²Gastroenterology Unit, Department of Medicine, Faculty of Medicine, Thammasat University Hospital

EXTERNAL LINK

https://doi.org/10.1371/journal.pone.0239434

THIS PROTOCOL ACCOMPANIES THE FOLLOWING PUBLICATION


DOI

dx.doi.org/10.17504/protocols.io.bigjkbun

EXTERNAL LINK

https://doi.org/10.1371/journal.pone.0239434

PROTOCOL CITATION


MANUSCRIPT CITATION  please remember to cite the following publication along with this protocol


LICENSE

This is an open access protocol distributed under the terms of the Creative Commons Attribution License, which permits unrestricted use, distribution, and reproduction in any medium, provided the original author and source are credited

CREATED

Jul 11, 2020

LAST MODIFIED

Oct 02, 2020

PROTOCOL INTEGER ID

39147
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% H2O2 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% H2O2 in 150 ml 0.05 M Tris pH 7.6)

11 **Stop the reaction by washing in PBS.**

12 **Counterstain with hematoxylin.**
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.

Coverslip slides using Permount (xylene based).