Protocol of Nrf2 staining form paraffin embedded human colon samples

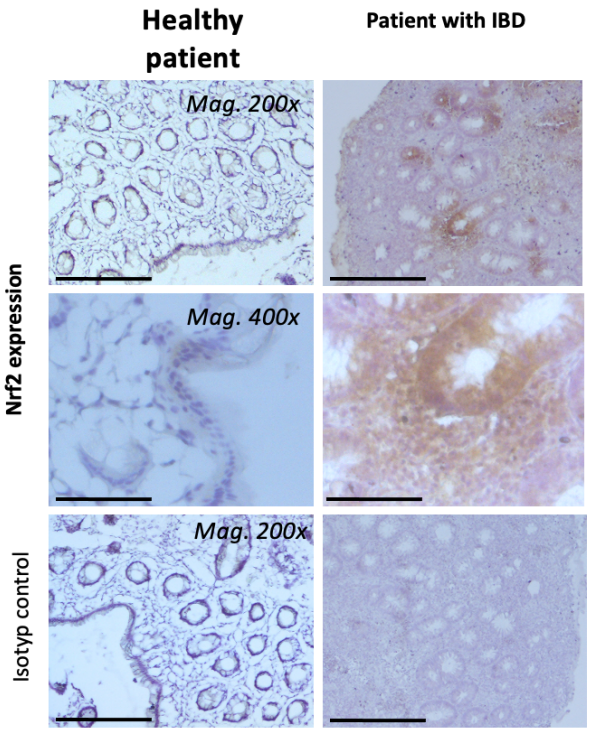
Reagents:

1. citrate buffer pH 6.0 (9 ml 0.1M citric acid + 41 ml 0.1M sodium citrate = set the pH to 6.0 and adjust the volume to 500 ml with distillated water)
2. Xylene
3. Ethanol 100%, 97%, 70%, 50%
4. Distillated H2O
5. Mouse and Rabbit Specific HRP/DAB (ABC) Detection IHC kit, Abcam
6. Mayer's hematoxylin

Protocol:

7. Cut samples to 6 μm on microtome.
8. Deparaffinize samples using two changes of the following: xylene (2 minutes each), 100% ethanol (2 minutes each), 97% ethanol (2 minutes each), 70% ethanol (2 minutes each), 50 % (2 minutes each) and distillated water.
9. Retrieve antigen in citrate buffer pH 6.0. Boil glass slides in a microwave. Each time stop when the boiling bubbles appear, wait 3 minutes, and repeat three times.
10. Cool on benchtop to room temperature (RT).
11. Wash samples once in dH2O for 5 min.
12. Circle samples with Pappen.
13. Pour few drops of hydrogen peroxide block (from ABC Detection IHC kit, Abcam) to each circle and incubate 10 minutes at RT.
14. Wash 2x in PBS without Ca^{2+} and Mg^{2+} for 5 min.
15. Pour few drops of protein block (from ABC Detection IHC kit, Abcam) to each circle and incubate 30 minutes at RT.
16. Wash 1x in PBS without Ca^{2+} and Mg^{2+} for 5 min.
17. Add I antibody for 1:50 Nrf2 rabbit polyclonal antibody (SantaCruz, H-300) in 3% goat serum in PBS without Ca^{2+} and Mg^{2+}. Incubate overnight at 4°C on wet chamber.
18. Wash 4x in PBS without Ca^{2+} and Mg^{2+} for 5 min.
19. Add II antibody provided in the ABC kit. Incubate one hour at RT on wet chamber.
20. Wash 4x in PBS without Ca^{2+} and Mg^{2+} for 5 min.
21. Add DAB mix (from the kit) and incubate for 5-10 minutes at RT.
22. Wash 4x in PBS without Ca^{2+} and Mg^{2+} for 5 min.
23. Counterstain with Mayer's hematoxilin for 1 minute
24. Wash slides in dH2O
25. Dehydrate samples in 70% ethanol (2 minutes each), 97% ethanol (2 minutes each), 100% ethanol (2 minutes each), xylene (2 minutes each).

Exemplary results:



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