Protocol for IHC-P
Instruction manual

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NOT FOR USE IN CLINICAL DIAGNOSTIC PROCEDURES

2nd Edition (Revised in April, 2018)

1. Baking: Bake the IHC slides for 0.5~2 hours at 60 Mark with a pencil and bake at 65°C for 1-2 hours.
2. Deparaffinization and rehydration: After the baking, put slides into the following reagents in order:
    1) Xylene I: 30 minutes
    2) Xylene II: 30 minutes
    3) 100% ethanol: 10 minutes
    4) 95% ethanol: 10 minutes
    5) 80% ethanol: 10 minutes
    6) 70% ethanol: 10 minutes
    7) Rinse sections with running water 10 minutes
3. Antigen repair: high temperature and high pressure method and microwave method.
   High temperature and high pressure: Sodium citrate antigen repair working solution(0.01M, pH6.0) about 1000ml into the pressure cooker. Put the slice into the cooker, cover the pot and preheat to boiling at 1600W. Then close the pressure valve at 1300W, start time when the valve jet, repair time stays 2 minutes.
   Microwave method: Put a certain amount of sodium citrate antigen repair working solution(0.01M, pH6.0) into microwave,and heat it to boiling. Then put the slice into the boiling buffer, handle with low fire for 3 minutes. Place it for 8 minutes and then handle with low fire for 3 minutes. Remove water and cool it, take the slice, wash with distilled water for twice.
4. Rinse 3X in PBST: Remove the residual, soak in PBST, and rinse in shaker at 40 RPM, 5min each time.
5 Blocking endogenous peroxidase: Incubate the slides with 3%-5% H_2O_2. Solution for 10min at room temperature.
6. Rinse 3X in PBST: Remove the residual, soak in PBST, and rinse in shaker at 40 RPM, 5min each time.

7. Blocking: Remove the residual, incubate the slide with 5% BSA solution for 20min at room temperature.
8. Incubation primary antibody: Remove the residual, incubate the slides with diluted primary antibody overnight at 4°C (recommended) or for 1 hour at 37°C.
9. Rewarming of slides: Move the slides from 4°C to room temperature for about 20min to avoid the off-slide for rinse immediately.
10. Rinse 3X in PBST: Remove the residual, soak in PBST, and rinse in shaker at 40 RAM, 5min each time.
11. Incubation with HRP-conjugated secondary antibody(SAA544Mu19 HRP-Linked Caprine Anti-Mouse IgG Polyclonal Antibody): Remove the residual, incubate the slides with diluted HRP-conjugated secondary antibody at a appropriate dilution for 60min at 4°C.
12. Rinse 3X in PBST: Remove the residual, soak in PBST, and rinse in shaker at 40 RAM, 5min each time.
13. Chromogenic detection: Use a DAB chromogenic kit (Cat # IS046) to stain the tissue section. Mix Reagent A and B thoroughly as the manual, then add appropriate volume to the tissue section and incubate at room temperature for 0.5-10 minutes. End the reaction by washing the tissue section with distilled water till a brown color develops.
14. Slightly counterstain the tissue section with haematoxylin.Then dry the tissue section, and put on a drop of resin seal the tissue section with a cover slip. The tissue section is ready for observation under a microscope.

Note:

1. The primary antibody concentration, incubation time and temperature directly affect the staining efficiency and background staining. If the positive staining is too weak, the concentration of the primary antibody and the incubation time can be increased; if the background is too high, the primary antibody concentration and the incubation time can be decreased. Such as wash the section with 0.01-0.02% TWEEN 20 PBS 4 times and with pure PBS twice before DAB staining, then use DAB chromogenic kit to stain the section.
2. PBS, TBS and other buffers can be used instead of the 0.01 M Citrate Buffer (pH6.0) in heat repair antigen process.
3. Dewax the tissue section thoroughly to avoid non-specificity background

staining occurred. It's recommended to separate the dewaxing in immunochemistry and in the H.E.