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Anti-Phospho-Ser^{261} AQP2 Antibody Immunohistochemistry Protocol

Catalog #: p112-261
Species: Rabbit
Tissue: Rat Kidney

Fixation: perfusion fixed with 3% paraformaldehyde in 0.1M cacodylate
Antibody incubation: Primary Antibody- 4C, overnight Secondary Antibody- RT, 2 hour

Materials Required

- ✓ **Fixative:** 3% Paraformaldehyde in freshly prepared 0.1M cacodylate buffer
- ✓ **0.1M Cacodylate buffer**: 21.4g of sodium cacodylate in 1L of ddH_2O, pH 7.4
- ✓ **graded ethanol:** 50% and 95% ethanol in dH2O
- ✓ **absolute methanol**
- ✓ **Eosin dye**
- ✓ **Xylene**
- ✓ **Paraffin**
- ✓ **Gelatin coated slides**
- ✓ **Endogenous peroxidase blocking solution:** 0.5% H2O2 in absolute methanol
- ✓ **Target Retrieval Solution:** 1mM Tris, pH 9.0, 0.5mM EGTA
- ✓ **1X PBS:** 137 mM NaCl, 28 mM Na_2HPO_4, 5.4 mM KCl, 2.9 mM KH_2PO_4 , pH 7.4
- ✓ **1X PBS blocking buffer:** 1% BSA, 0.05% saponin, 0.2% gelatin
- ✓ **1X PBS wash buffer:** 0.1% BSA, 0.05% saponin, 0.2% gelatin
- ✓ **Nonspecific Blocking buffer:** 50mM NH_4Cl in 1X PBS
- ✓ **Incubation buffer:** 1X PBS with 0.1% BSA, 0.3% Triton X-100
- ✓ **Secondary Antibody:** example used is goat anti-rabbit HRP from Agilent-DAKO (catalog # P044801-2)
- ✓ **DAB:** 0.05% 3,3' –diaminobenzidine tetrachloride dissolved in dH_2O with 0.1% H_2O_2 (Sigma catalog # D5637)
- ✓ **Mounting media:** Non-polar, hydrophobic mounting media Eukitt UV (Sigma catalog # 05393)

Protocol

1. Perfusion fix animal through the abdominal aorta. Flush kidneys with cold 1x PBS (pH 7.4) for 15 seconds to remove blood, then switch to cold fixative for 3 minutes.
2. Remove kidneys and section the mid-region into 2-3mm transverse sections. Immerse sections in fixative for an additional hour.

   Tech Tip:

   a. Fixative volume must 20x of the tissue size for proper fixation due to organ density and thickness. If not done properly, inadequate penetration of fixative occurs and tissue will be soft and will not stain.

3. Wash tissue sections with 0.1M cacodylate buffer 3 times, in 10 minute intervals.
4. Dehydrate tissue sections in graded ethanol (50/95/95), then leave in xylene overnight.
   Tech Tip:
   a. Avoid long periods of dehydration in 95% ethanol. This can cause excessive shrinkage and hardening of the tissue sections.
   b. Eosin can be added to the ethanol to dye small colorless tissue sections pink so they can be seen and sectioned when embedded in paraffin.
5. Embed tissue sections in paraffin and cool to room temperature.
6. Cut the embedded tissue into 2-µm sections with a rotary microtome. Mount tissue sections onto gelatin coated slides. Dry sections overnight at 37C or at room temperature.
7. Dewax the tissue sections with xylene and rehydrate with graded ethanol.
8. Block endogenous peroxidase activity with 0.5% H_2O_2 in absolute methanol for 10 minutes.
9. Perform antigen retrieval by microwave boiling sections in target retrieval solution for 10 minutes.
10. Nonspecifically block the sections with 50mM NH_4Cl in 1xPBS for 30 minutes.
11. Wash the sections with 1x PBS blocking buffer 3 times, in 10 minute intervals.
12. Dilute Anti-Phospho-Ser^{261} AQP2 antibody (Cat. # p112-261) to 1:2000 in incubation buffer. Incubate sections overnight at 4C.
13. Remove primary antibody solution and wash the slides with 1X PBS washing buffer 3 times, in 10 minute intervals.
14. Dilute secondary antibody in secondary incubation buffer per manufacturer's recommendation. Incubate sections for 1 hour at room temperature.
    Tech Tip:
    a. HRP-conjugated secondary antibody was used to produce the images.
15. Remove secondary antibody and wash with 1x PBS wash buffer 3 times, in 10 minute intervals.
16. Apply DAB solution to the tissue section. Incubate for no longer than 10 minutes.
    Tech Tip:
    a. Once DAB solution is applied watch until optimal staining has occurred. Do not walk away until stain is satisfactory, as over staining may occur.
17. Rinse slides with 1x PBS and apply hematoxylin for counter staining.
18. Dehydrate section and apply mounting medium onto slide and gently place glass cover slip before viewing under the microscope.
    Tech Tip:
    a. The cover slips were mounted with a hydrophobic medium to preserve the tissue section stains.

Reference:

Hoffert JD, Nielsen J, Yu MJ, Pisitikun T, Schleicher SM, Nielsen S, Knepper MA (2007) Dynamics of aquaporin-2 serine-261 phosphorylation in response to short-term vasopressin treatment in collecting duct. *Am J Physiol Renal Physiol* 292: F691-F700.