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RABBIT ANTI-ROMK1
(Inwardly Rectifying Potassium Channel)
POLYCLONAL ANTIBODY

CATALOG NUMBER: AB5196

LOT NUMBER:

QUANTITY: 200 µL

CONCENTRATION: 0.8 mg/mL (after reconstitution)

SPECIFICITY: Recognizes a full length ROMK1 protein. Does not cross react with any other potassium channel antigens tested so far.

IMMUNOGEN: GST fusion protein and a C-terminal portion of rat ROMK1 protein (amino acids 342-391) (Accession P35560).

APPLICATIONS: Western blot: 1:200-1:400 using ECL on rat kidney membranes.
Immunohistochemistry on rat kidney tissue.
Dilutions should be made using a carrier protein such as BSA (1-3%)
Optimal working dilutions must be determined by the end user.

CONTROL ANTIGEN: Included free of charge with the antibody is 120 µg of control antigen (lyophilized powder). The stock solution of the antigen can be made up using 100 µL of PBS. For positive control, in Western blot using 20 ng of protein per minigel lane. For negative control, preincubate 3 µg of fusion protein with 1 µg of antibody for one hour at room temperature. Optimal concentrations must be determined by the end user.

SPECIES REACTIVITIES: Rat and mouse. Other species have not been tested.

FORMAT: Affinity purified immunoglobulin.

PRESENTATION: Lyophilized from phosphate buffered saline, pH 7.4, containing 1% BSA, and 0.025% sodium azide as a preservative. Reconstitute with 200 µL of sterile deionized water. Centrifuge antibody preparation before use (10,000 xg for 5 min).

STORAGE/HANDLING: Maintain lyophilized material at -20°C for up to 12 months. After reconstitution maintain at -20°C in undiluted aliquots for up to 6 months. Avoid repeated freeze/thaw cycles.

For research use only; not for use as a diagnostic.

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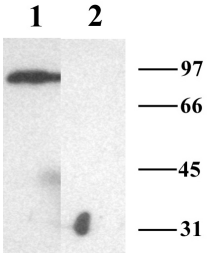
SUGGESTED WESTERN BLOT PROTOCOL

1. Mix the samples (organ membranes: 50 µg/lane; transfected cells: 500,000 cells/lane) with sample-buffer X 2, and heat 10 min at 70°C.
2. 5-50 µL applied to Minigel lane (0.75-1.5 mm width) and run at standard conditions. (60 mA for 2 1.5 mm Minigel gels, 1.4 h). It is suggested that you run 5-15% acrylamide (37.5:1 acrylamide:bisacrysmide) minigel (1.5 mm width) at 30 mA/gel ~1-1.5 hours.
3. Transfer in semi-dry system under standard conditions (3 h 100 mA for two minigel gels)
4. Stain the transferred bands with Chemicon BLOT-*FastStain* (Catalog Number 2076).
5. Destain with deionized water.
6. Block with 5% non-fat milk (Marvel or Carnation) in PBS, and 0.025 % sodium azide, overnight at 2-8°C. The non-fat milk should be dissolved freshly, centrifuged 10,000 rpm for 10 min, and filtered through glass filter (Gelman Acrodisc).
7. Incubation with first antibody 2 h at room temperature or overnight at 4°C in blocking solution. The antibody preparation should be centrifuged before use (10,000 g 5 min.). Optimal working dilutions and incubation time will need to be determined by the end user.
8. Wash 4 x 10 min. with PBS-0.1% tween 20. From this stage, azide should be omitted.
9. Incubation with the secondary antibody (HRP-conjugated goat anti-rabbit antibody, for example Chemicon Catalog Number AP132P, diluted appropriately) 1 h at room temperature.
10. Wash 4 x 10 min. with PBS-0.1% tween 20.
11. Perform ECL with commercial kits (*Chemi*LUCENT, Chemicon Catalog Number 2600).



Western blotting of rat kidney membranes:
1. AB5196 (1:200)
2. AB5196, preincubated with the control antigen.

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