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RABBIT ANTI-AQUAPORIN 3
AFFINITY PURIFIED
POLYCLONAL ANTIBODY

CATALOG NUMBER: AB3276-200UL

LOT NUMBER:

QUANTITY: 200 µL

CONCENTRATION: 0.4 mg/mL (after reconstitution)

SPECIFICITY: Recognizes Aquaporin 3 (AQP3, GLIP).

IMMUNOGEN: Highly purified peptide corresponding to residues 275-292 of rat Aquaporin 3 (Accession number P47862).

APPLICATIONS: Western blotting: 1:200 on rat kidney membranes.
Immunohistochemistry on mouse kidney sections.
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 XX µg of control antigen (lyophilized powder). Reconstitute with 100 µL of deionized water. For negative control, preincubate 1 µg of purified peptide 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. Reactivity with other species has not yet been tested. The epitope specific for Aquaporin 3 is highly homologous in human and sheep (15/18).

FORMAT: Affinity purified immunoglobulin.

PRESENTATION: Lyophilized from PBS, pH 7.4, containing 1% BSA, and 0.025% sodium azide. Reconstitute with 200 µL of sterile distilled water. Centrifuge antibody preparation before use (10,000 x g for 5 min).

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

REFERENCE: Mobasheri, A., et al., (2004). *Vet J.* 168(2):143-150.

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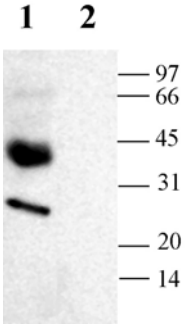
SUGGESTED WESTERN BLOT PROTOCOL

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 (Chemilucent, Chemicon Catalog Number 2600).



Western blotting of rat kidney membranes (25 μg/lane):
1. AB3276 (1:200)
2. AB3276, preincubated with the control peptide antigen

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