



## RABBIT ANTI-AQUAPORIN 2 AFFINITY PURIFIED POLYCLONAL ANTIBODY

<b>CATALOG NUMBER:</b>	AB3274-50UL
<b>LOT NUMBER:</b>	
<b>QUANTITY:</b>	50 $\mu$ L
<b>CONCENTRATION:</b>	0.8 mg/mL (after reconstitution)
<b>SPECIFICITY:</b>	Recognizes Aquaporin 2 (AQP2, AQP-CD, WCH-CD).
<b>IMMUNOGEN:</b>	Highly purified peptide corresponding to residues 254-271 of rat or mouse Aquaporin 2 (SwissProt accession number P34080).
<b>APPLICATIONS:</b>	Western blotting: 1:200-1:600 using ECL on rat kidney membranes. Immunohistochemistry on rat 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.
<b>CONTROL ANTIGEN:</b>	Included free of charge with the antibody is XX $\mu$ g of control antigen (lyophilized powder). Reconstitute with 100 $\mu$ L of deionized water. For negative control, preincubate 1 $\mu$ g of purified peptide with 1 $\mu$ g of antibody for one hour at room temperature. Optimal concentrations must be determined by the end user.
<b>SPECIES REACTIVITIES:</b>	Human and rat. Reactivity with other species has not yet been tested. The epitope specific for Aquaporin 2 is identical in rat and mouse and only slightly different in human and sheep (17/18 amino acids).
<b>FORMAT:</b>	Affinity purified immunoglobulin.
<b>PRESENTATION:</b>	Lyophilized from PBS, pH 7.4, containing 1% BSA and 0.05% sodium azide. Reconstitute with 50 $\mu$ L of sterile distilled water. Centrifuge antibody preparation before use (10,000 x g for 5 min).
<b>STORAGE/HANDLING:</b>	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.

## 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:bisacrylamide) 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 MILLIPORE 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 MILLIPORE 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 (Chemiluminescent, MILLIPORE Catalog Number 2600).

**Important Note:** *During shipment, small volumes of product will occasionally become entrapped in the seal of the product vial. For products with volumes of 200 µL or less, we recommend gently tapping the vial on a hard surface or briefly centrifuging the vial in a tabletop centrifuge to dislodge any liquid in the container's cap.*

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