



PhosphoSolutions®
Antibodies that work™

Colorado Biosciences Park
12635 East Montview Boulevard, #213
Aurora, CO 80045-7337
Tel: (888) 442-7100

Anti-Phospho-Ser²⁶¹ Aquaporin 2

Catalog Number: p112-261

Size: 100 µl

Product Description: Affinity purified rabbit polyclonal antibody

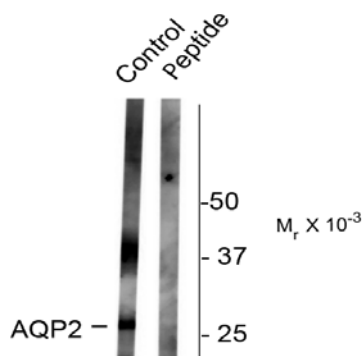
Applications: WB: 1:1000

Antigen: Phosphopeptide corresponding to amino acid residues surrounding the phospho-Ser²⁶¹ of rat aquaporin 2.

Species reactivity: The antibody has been directly tested for reactivity in Western blots with rat tissue. It is anticipated that the antibody will react with canine, bovine, chicken, human, mouse and non-human primate tissues based on the fact that these species have 100% homology with the amino acid sequence used as antigen.

Biological Significance: Aquaporin 2 (AQP2) is a hormonally regulated water channel located in the renal collecting duct. Mutations in the AQP2 gene cause hereditary nephrogenic diabetes insipidus in humans (Iolascon et al., 2007). A vasopressin induced cAMP increase results in the phosphorylation of AQP2 at serine-256 and its translocation from the intracellular vesicles to the apical membrane of principal cells (van Balkom et al., 2002). Recently, serine-261 has been identified as a novel phosphorylation site on AQP2 and levels of phosphorylated S261 have been shown to decrease with vasopressin treatment suggesting its involvement in vasopressin-dependent AQP2 trafficking (Hoffert et al., 2007).

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Western blot of rat kidney lysate showing specific immunolabeling of the ~ 29k and 37k glycosylated form of the AQP2 protein phosphorylated at Ser²⁶¹. Immunolabeling is blocked by the phospho-peptide used as antigen (peptide) but not by the corresponding dephospho-peptide (not shown).

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WB = Western Blot **IF** = Immunofluorescence **IHC** = Immunohistochemistry **IP** = Immunoprecipitation

Packaging: 100 µl in 10 mM HEPES (pH 7.5), 150 mM NaCl, 100 µg per ml BSA and 50% glycerol. Adequate amount of material to conduct 10-mini Western Blots.

Storage and Stability: Store at -20°C; stable for at least one year.

Shipment: Domestic - Blue Ice; International - Blue Ice or Dry Ice.

Purification Method: Prepared from rabbit serum by affinity purification via sequential chromatography on phospho- and dephosphopeptide affinity columns.

Antibody Specificity: Specific for ~29k AQP2 protein phosphorylated at Ser²⁶¹. Also recognizes the glycosylated form of AQP2 at ~ 37k. Immunolabeling of the AQP2 band is blocked by preadsorption with the phospho-peptide used as antigen but not by the corresponding dephospho-peptide.

Quality Control Tests: Western blots performed on each lot.

References:

- van Balkom BW, Savelkoul PJ, Markovich D, Hofman E, Nielsen S, van der Sluijs P, Deen PM (2002) The role of putative phosphorylation sites in the targeting and shuttling of the aquaporin 2 water channel. *J Biol Chem* 277(44):41473-9.
- Ford P, Rivarola V, Chara O, Blot-Chabaud M, Cluzeaud F, Farman M, Parisi M, Capurro C (2005) Volume regulation in cortical collecting duct cells: role of AQP2. *Biol Cell* 97(9):687-97.
- Hoffert JD, Nielsen J, Yu MJ, Pisitkun T, Schleicher SM, Nielsen 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.
- Iolascon A, Aglio V, Tamma G, D'Appolito M, Addabbo F, Procino G, Simonetti MC, Montini G, Gesulado L, Debler EW, Suelto M, Valenti G (2007) Characterization of two novel missense mutations in AQP2 gene causing nephrogenic diabetes insipidus. *Nephron Physiol.* 105(3): p33-41.

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