anti-Actinin 4

affinity purified rabbit antibody IG701

Lot: 581 REV: 110501 catalog #: 0042-05



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Background information

 $\alpha\text{-}Actinin~4$ is an actin-bundling protein of ~100 kDa that is associated with cell motility, endocytosis, and cancer invasion [1, 2]. The $\alpha\text{-}Actinin~$ family comprises two non-muscle isoforms ($\alpha\text{-}Actinin~$ 1 and 4) and two skeletal muscle isoforms ($\alpha\text{-}Actinin~$ 2 and 3), with $\alpha\text{-}Actinin~$ 2 being also expressed in cardiac muscle [3].

While α -Actinin 4 is almost ubiquitously expressed, particularly high concentrations are found in glomeruli. On the subcellular level it is associated with actin stress fibers, but in certain cells it also localizes to the nucleus [1].

Mutations in the α -actinin 4 gene cause an autosomal-dominant form of familial focal segmental glomerulosclerosis (FSGS) [4], which is thought to result from a defect in glomerular podocyte function. A point mutation in the α -actinin 4 gene was found to generate an antigenic peptide that is recognized by autologous cytolytic T lymphocytes (CTL) on a human lung carcinoma [5].

 α -Actinin 4 interacts with a variety of proteins, including the ring finger protein BERP [6], the protein CLP-36 PDZ-LIM [7]. hemidesmosomal and cell-cell contact protein BP180 [8], and the tight junction protein MAGI-1 [9]. Moreover, α -Actinin 4 forms a ternary Ca²⁺/Calmodulin-dependent with complex protein kinase II and Densin-180, a protein of postsynaptic densities in CNS neurons [10]. Ca^{2+} -dependent association of α -Actinin 4 with E3KARP is required for Ca²⁺-dependent inhibition of the Na⁺/H⁺ exchanger 3 (NHE3) [11].

Antibody preparation and storage

200 μ l of purified antibody in PBS containing 1 mg/ml BSA, 0.01% (w/v) NaN3. Antibody concentration: 250 μ g/ml. Vials have been overfilled by 10% to ensure complete recovery of the specified amount. Short term storage at 4°C, stable for one year from date of shipment when stored at -20°C. Avoid repeated freezing and thawing! Do <u>not</u> store in "frost-free" freezers.

Antigen

The antibody was raised as described [1] and is directed against a synthetic peptide from the N-terminal part of Actinin 4 (MGDYMAQEDDW) coupled to KLH via a C-terminal cysteine residue. The antibody was affinity purified on the sepharose bound peptide.

Species and Actinin isoform cross-reactivity

By reaction with a set of solid phase bound synthetic peptides, representing the homologous regions of different α -Actinin isoforms, the antibody was shown to be highly specific for Actinin 4 as compared to Actinins 1-3 (Exp. Conditions: 1:10,000 dilution of IG701, enhanced chemoluminescence detection):

Spot #: 1 2 3 4 5 6

Sequence

Isoform (Species)

1 QTNDYMQPEEDWD Actinin 1 (human, mouse, rat, chicken)
2 DEDEYMIQEEEWD Actinin 2 (human, mouse)
3 GGGEYMEQEEDWD Actinin 3 (human, mouse, rat)
4 SMGDYMAQEDDWD Actinin 4 (human, mouse, rat)
5 GMGDYMRPGGDWD Actinin 4 variant (rat)
6 MDAWYSDEQMDGD Control (scrambled Actinin 4 peptide)

Species cross-reactivity in immunoblots of total cell or tissue lysates: human, mouse, rat, chinese hamster, pig

Applications

Immunoblotting (0.1-0.25 μ g/ml; 1:1,000 - 1:2,500), immuno-fluorescence (1 μ g/ml; 1:250), immunoprecipitation (25 μ g/ml; 1:10). The recommended dilutions refer to the analysis of mouse cells and tissues with intermediate levels of Actinin 4 expression and must be viewed as approximate.

Positive control

Human platelet protein (500 μ g), supplied at 5 mg/ml in SDS sample buffer. Use 5 μ l (25 μ g) per lane for Western blotting.

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Related products

- affinity purified rabbit antibody IG731 to human VASP, 25 μg (catalog # 0012-02)
- monoclonal antibody IE273 to human VASP, 50 μg (catalog # 0016-05)
- affinity purified rabbit antibody to human LPP, 50 μg (catalog # 0032-05)
- affinity purified rabbit antibody to profilin, 10 μg (catalog # 022-01)

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