

p-Adducin (Ser 662): sc-12614

BACKGROUND

Adducins are a family of cytoskeleton proteins encoded by three genes (α , β and γ). Adducin is a protein associated with the inner leaflet of the plasma membrane and is one of the proteins localized at the spectrin-Actin junction of the membrane skeleton. Adducins promote association of spectrin with Actin and cap the fast growing end of Actin filaments. Adducins contain an N-terminal core, neck and C-terminal tail domains, are substrates for protein kinase A (PKA) and C (PKC), and bind to Ca^{2+} /calmodulin. The major phosphorylation sites common to the Adducins are Ser 726 and Ser 713 in the C-terminal MARCKS-related domains of Adducin α and Adducin β , which are phosphorylated by PKA and PKC, respectively. In addition, PKA phosphorylates Adducin α at Ser 408, 436 and 481. Calmodulin-binding is inhibited by phosphorylation of Adducin β , but not Adducin α . Rho-kinase can phosphorylate Adducin α at Thr 445 and Thr 480 downstream of Rho *in vivo*. The phosphorylation of Adducin by Rho-kinase plays an important role in the regulation of membrane ruffling and cell motility. In addition, phosphorylation at Ser 726 of Adducin α is required for cleavage by caspase-3.

REFERENCES

1. Matsuoka, Y., et al. 1996. Adducin regulation. Definition of the calmodulin-binding domain and sites of phosphorylation by protein kinases A and C. *J. Biol. Chem.* 271: 25157-25166.
2. Fukata, Y., et al. 1999. Phosphorylation of Adducin by Rho-kinase plays a crucial role in cell motility. *J. Cell Biol.* 145: 347-361.
3. Gilligan, D.M., et al. 1999. Targeted disruption of the β Adducin gene (Add2) causes red blood cell spherocytosis in mice. *Proc. Natl. Acad. Sci. USA* 96: 10717-10722.
4. Muro, A.F., et al. 2000. Mild spherocytic hereditary elliptocytosis and altered levels of α and γ Adducins in β Adducin-deficient mice. *Blood* 95: 3978-3985.
5. van de Water, B., et al. 2000. Cleavage of the Actin-capping protein α Adducin at Asp-Asp-Ser-Asp633-Ala by caspase-3 is preceded by its phosphorylation on Serine 726 in cisplatin-induced apoptosis of renal epithelial cells. *J. Biol. Chem.* 275: 25805-25813.

SOURCE

p-Adducin (Ser 662) is available as either goat (sc-12614) or rabbit (sc-12614-R) polyclonal affinity purified antibody raised against a short amino acid sequence containing Ser 662 phosphorylated Adducin γ of human origin.

PRODUCT

Each vial contains 200 μ g IgG in 1.0 ml of PBS with < 0.1% sodium azide and 0.1% gelatin.

Blocking peptide available for competition studies, sc-12614 P, (100 μ g peptide in 0.5 ml PBS containing < 0.1% sodium azide and 0.2% stabilizer protein).

APPLICATIONS

p-Adducin (Ser 662) is recommended for detection of Adducin γ phosphorylated at Ser 662, Adducin α phosphorylated at Ser 724 and Adducin β phosphorylated at Ser 713 of mouse, rat and human origin by Western Blotting (starting dilution 1:200, dilution range 1:100-1:1000), immunoprecipitation [1-2 μ g per 100-500 μ g of total protein (1 ml of cell lysate)], immunofluorescence (starting dilution 1:50, dilution range 1:50-1:500) and solid phase ELISA (starting dilution 1:30, dilution range 1:30-1:3000).

p-Adducin (Ser 662) is also recommended for detection of correspondingly phosphorylated Adducin γ , Adducin α and Adducin β in additional species, including equine, canine, bovine, porcine and avian.

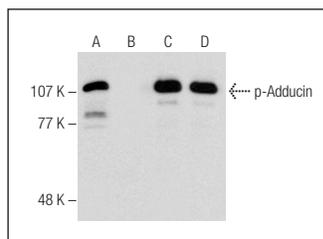
Molecular Weight of p-Adducin α : 120 kDa.

Molecular Weight of p-Adducin β : 97 kDa.

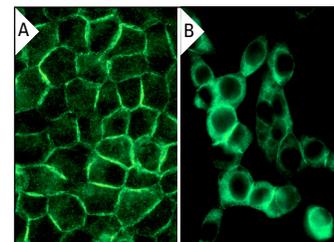
Molecular Weight of p-Adducin γ : 94 kDa.

Positive Controls: K-562 whole cell lysate: sc-2203, NIH/3T3 whole cell lysate: sc-2210 or mouse brain extract: sc-2253.

DATA



Western blot analysis of Adducin phosphorylation in untreated (A, C) and lambda protein phosphatase (sc-200312A) treated (B, D) HL-60 whole cell lysates. Antibodies tested include p-Adducin (Ser 662)-R: sc-12614-R (A, B) and Adducin α (H-100): sc-25731 (C, D).



p-Adducin (Ser 662): sc-12614. Immunofluorescence staining of methanol-fixed HeLa cells (A) and NIH/3T3 cells (B) showing cytoskeletal localization.

STORAGE

Store at 4° C, ****DO NOT FREEZE****. Stable for one year from the date of shipment. Non-hazardous. No MSDS required.

RESEARCH USE

For research use only, not for use in diagnostic procedures.

PROTOCOLS

See our web site at www.scbt.com or our catalog for detailed protocols and support products.