



PhosphoSolutions®
Antibodies that work™

Colorado Biosciences Park
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Anti-Phospho-Ser⁷⁷⁴ Dynamin

Catalog Number: p1050-774

Size: 100 µl

Product Description: Affinity purified sheep polyclonal antibody

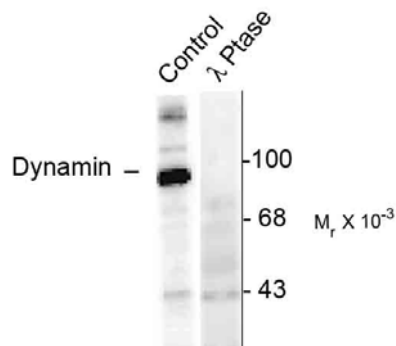
Applications: **WB:** 1:1000
IF (fixed cultured neurons; Tan et al., 2003): 1:1000
IHC (fixed cultured neurons, Tan et al., 2003): 1:1000

Antigen: Phosphopeptide corresponding to amino acid residues surrounding the phospho-Ser⁷⁷⁴ of rat dynamin.

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

Biological Significance: Dynamin is a member of a group of nerve terminal proteins called dephosphins that regulate synaptic vesicle endocytosis (Cousin et al., 2001; Graham et al., 2002; Tsuboi et al., 2002). Cyclin dependent protein kinase 5 phosphorylates dynamin at Ser⁷⁷⁴ and Ser⁷⁷⁸ that are the phosphorylation sites on dynamin phosphorylated *in vivo* (Tan et al., 2003). Phosphorylation of Ser⁷⁷⁴ by GSK3 has recently been shown to control activity-dependent bulk endocytosis of synaptic vesicles (Clayton et al., 2010)

Anti-Phospho Ser⁷⁷⁴ Dynamin



Western blot of rat hippocampal lysate stimulated with forskolin showing specific immunolabeling of the ~95k dynamin phosphorylated at Ser⁷⁷⁴ (Control). The phosphospecificity of this labeling is shown in the second lane (*lambda*-phosphatase: λ-Ptase). The blot is identical to the control except that it was incubated in λ-Ptase (1200 units for 30 min) before being exposed to the phospho-Ser⁷⁷⁴ dynamin. The immunolabeling is completely eliminated by treatment with λ-Ptase.

Page 1 of 2

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

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

Storage and Stability. For long term storage -20°C is recommended. Stable at -20°C for at least 1 year.

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

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

Antibody Specificity: Specific for the ~95k dynamin protein phosphorylated at Ser⁷⁷⁴. Labels the purified protein phosphorylated *in vitro* by cdk5 but not by PKC. Does not cross react with other purified substrates of cdk5 (e.g. amphiphysin and synapsin). Immunolabeling is blocked by λ -phosphatase treatment.

Quality Control Tests: Western blots performed on each lot.

References:

- Cousin MA, Tan TC, Robinson PJ (2001) Protein phosphorylation is required for endocytosis in nerve terminals: potential role for the dephosphins dynamin I and synaptojanin, but not AP180 or amphiphysin. *J Neurochem* 76:105-116.
- Graham ME, O'Callaghan DW, McMahon HT, Burgoyne RD (2002) Dynamin-dependent and dynamin-independent processes contribute to the regulation of single vesicle release kinetics and quantal size. *Proc Natl Acad Sci USA* 99:7124-7129.
- Tan TC, Valova VA, Malladi CS, Graham ME, Berven LA, Jupp OJ, Hansra G, McClure SJ, Sarcevic B, Boadle RA, Larsen MR, Cousin MA, Robinson PJ (2003) cdk5 is essential for synaptic vesicle endocytosis. *Nat Cell Biol* 5:701-710.
- Tsuboi T, Terakawa S, Scalettar BA, Fantus C, Roder J, Jeromin A (2002) Sweeping model of dynamin activity - Visualization of coupling between exocytosis and endocytosis under an evanescent wave microscope with green fluorescent proteins. *J Biol Chem* 277:15957-15961.
- Clayton EL, Sue N, Smillie KJ, O'Leary T, Bache N, Cheung G, Cole AR, Wyllie DJ, Sutherland C, Robinson PJ, Cousin MA. (2010) Dynamin I phosphorylation by GSK3 controls activity-dependent bulk endocytosis of synaptic vesicles. *Nat Neurosci.* 13(7):845-51.
- Sergio Leal-Ortiz, Clarissa L. Waites, Ryan Terry-Lorenzo, Pedro Zamorano, Eckart D. Gundelfinger, and Craig C. Garner (2008) Piccolo modulation of Synapsin1a dynamics regulates synaptic vesicle exocytosis. *J. Cell Biol.*, 181: 831 - 846.

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