

Anti-Phospho-Ser⁷⁷⁴ Dynamin Antibody



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Host	Applications	Species Tested	Species Reactivity*	Molecular Reference
Sheep	WB 1:1000 IF 1:1000 (Tan et al., 2003) IHC 1:1000 (Tan et al., 2003)	R	B, C, Ch, H, M, NHP	~95 kDa

Product Description: Affinity purified sheep polyclonal antibody.

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).

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

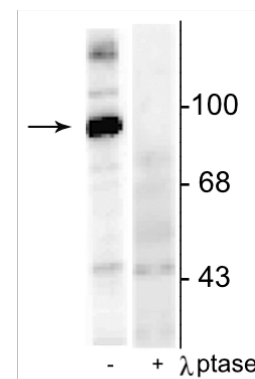
Antibody Specificity: Specific for endogenous levels of the ~95 kDa dynamin protein phosphorylated at Ser⁷⁷⁴. Labels the purified protein phosphorylated *in vitro* by cdk5 but not by PKC. Does not cross react with other purified substrates of cdk5 (e.g. amphiphysin and synapsin). Immunolabeling is blocked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

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

Quality Control Tests: Western blots performed on each lot.

Packaging: 100 µl in 10 mM HEPES (pH 7.5), 150 mM NaCl, 100 µg BSA per ml and 50% glycerol.

Storage and Stability: Shipped on blue ice. Storage at -20°C is recommended, as aliquots may be taken without freeze/thawing due to presence of 50% glycerol. Stable for at least 1 year at -20°C.



Western blot of rat hippocampal lysate stimulated with forskolin showing specific immunolabeling of the ~95 kDa dynamin phosphorylated at Ser⁷⁷⁴ in the first lane (-). Phosphospecificity is shown in the second lane (+) where immunolabeling is completely eliminated by blot treatment with lambda phosphatase (λ-Ptase, 1200 units for 30 min).

Product Specific References:

Vargas, K.J., Schrod, N., Davis, T., Fernandez-Busnadiego, R., Taguchi, Y.V., Laugks, U., Lucic, V. and Chandra, S.S., 2017. Synucleins Have Multiple Effects on Presynaptic Architecture. *Cell Reports*, 18(1), pp.161-173.

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.

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.

General 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.

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.