

Background

Members of the Wiskott-Aldrich syndrome protein (WASP) family regulate the formation of actin-based cell structures in many cell types. These proteins contain C-terminal actin-binding domains that can stimulate actin polymerization. WASP is expressed primarily in hematopoietic cells, while its homolog N-WASP is widely expressed. These proteins have 48% identity in human with higher homology in the functional regions of these proteins. Phosphorylation at serine and tyrosine residues regulates the activity of both proteins. WASP is tyrosine phosphorylated at tyrosine 291 after antigen receptor activation in B-cells and collagen stimulation of platelets. Phosphorylation of the analogous site in N-WASP (Tyr-256) stimulates its activity, reduces nuclear N-WASP, and is required for neurite extension.

Background References

Baba, Y. et al. (1999) *Blood* 93:2003.
Torres, E. & Rosen, M.K. (2003) *Mol Cell* 11:1215.
Wu, X. et al. (2004) *J Biol Chem* 279(10):9565.

Product Citations

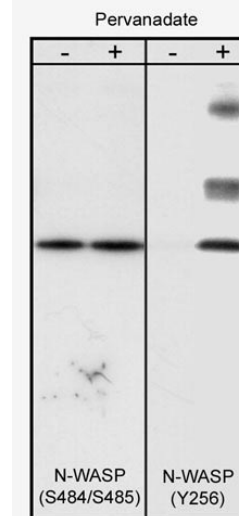
Elias, BC et al. (2015) *J Cell Sci.* 128(23):4293.
WB: mouse kidney duct cells

Uenishi, E. et al. (2013) *J of Bio Chem* 288(36): 25851.
WB: MIN6-K8 β-cells

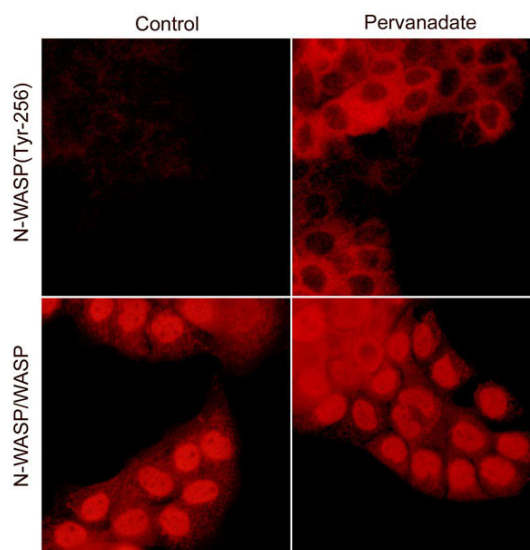
Cheney, L. et al. (2011) *J Infectious Diseases.* 203:1824.
WB: mouse adipocytes

Kalwa, H. & Michel, T. (2011) *J Biol Chem* 286:2320.
WB: bovine aortic endothelial cells

Pichot, C.S. et al. (2009) *British J Cancer.* 1 –10.
WB: breast cancer cell lines



Western blot analysis of control and pervanadate-treated A431 cells (20 µg/lane). Blots were probed with either rabbit polyclonal anti-N-WASP (Ser-484/Ser-485) or anti-N-WASP (Tyr-256).



Immunocytochemical labeling of N-WASP in control and pervanadate-treated A431 cells. The cells were labeled with rabbit polyclonal N-WASP/WASP (WP2101) or rabbit polyclonal N-WASP (Tyr-256) antibodies, then the antibodies were detected using appropriate secondary antibody conjugated to DyLight® 594.

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Immunogen

Uniprot ID: O00401

Phospho-N-WASP (Tyr-256) synthetic peptide (coupled to BSA) corresponding to amino acid residues around tyrosine 256 of human N-WASP. The human WASP sequence has a two amino acid difference in the same region surrounding tyrosine 291.

Buffer and Storage

Rabbit polyclonal, affinity-purified antibody is supplied in 100µl phosphate-buffered saline, 50% glycerol, 1 mg/ml BSA, and 0.05% sodium azide. Store at -20°C. Stable for 1 year.

Applications

WB	1:1000
ELISA	1:2000
ICC	1:100

Species Reactivity

Hu, Rt, Ms

End user should determine optimal dilution for their particular applications and experiments.

Western blot membranes were incubated with diluted antibody in 5% non-fat milk, Tris buffer, 0.04% Tween20 for 1 hour at room temperature.

Abbreviations: E = ELISA, ICC = immunocytochemistry, IHC = immunohistochemistry, IP = immunoprecipitation, MS = mass spectrometry, WB = western blot
Hu = Human, Ms = Mouse, Rt = Rat, Ck = Chicken, F = Frog, B = Bovine

Specificity

This antibody was cross-adsorbed to phosphotyrosine then affinity purified using phospho-N-WASP (Tyr-256) peptide (without carrier). The antibody detects a 65 kDa* protein corresponding to the molecular mass of phosphorylated N-WASP on SDS-PAGE immunoblots of A431 cells treated with pervanadate. A similar band is observed in pervanadate treated HeLa and endothelial cells. Weak bands are also observed at higher molecular weights after pervanadate treatment. These bands may be due to low cross-reactivity with phosphotyrosine.

*All molecular weights (MW) are confirmed by comparison to MW standards and to western blot mobilities of known proteins with similar MW.

"Native" western blot utilizes non-reducing sample buffer (no mercaptoethanol or SDS), normal SDS-PAGE gel electrophoresis, and no methanol in transfer buffers.

Related Products

WP2401 unphosphorylated N-WASP (Ser-484/Ser-485) Rabbit Polyclonal

WP2201 N-WASP (Ser-484/Ser-485), phospho-specific Rabbit Polyclonal

WP2001 N-WASP Rabbit Polyclonal

WP2101 WASP / N-WASP Rabbit Polyclonal

WX2605 phospho-N-WASP (Tyr-256) Blocking Peptide

WK6130 WAVE Phospho-Regulation Antibody Sampler Kit

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