SANTA CRUZ BIOTECHNOLOGY, INC.

N-WASP siRNA (m): sc-36007

BACKGROUND

The Wiskott-Aldrich syndrome (WAS) is characterized by thrombocytopenia, eczema, defects in cell-mediated and humoral immunity, and a propensity for lymphoproliferative diseases. The syndrome is the result of a mutation in the gene encoding a proline-rich protein termed WASP. WASP and the related protein neural-WASP (or N-WASP) are downstream effectors of Cdc42. Both WASP and N-WASP are implicated in Actin polymerization and cytoskeletal organization, and N-WASP is also essential for mediating the Cdc42-induced formation of filopodia. WASP is primarily expressed in hematopoietic cells, whereas N-WASP is richest in neural tissues and is also expressed ubiquitously. The effects of Cdc42-stimulated Actin assembly require the interaction of WASP/N-WASP with the Arp2/3 complex, which dramatically enhances polymerization. The WASP and N-WASP proteins characteristically contain a pleckstrin homology (PH) domain, which binds phosphatidyl-inositol bisphosphate (PIP2); a Cdc42-binding domain; and a 70 amino acid conserved verprolin-homology (VPH) domain, which is the Actin-binding region and is critical to the regulation of the Actin cytoskeleton.

REFERENCES

1. Remold-O'Donnell, E., et al. 1996. Defects in Wiskott-Aldrich syndrome blood cells. Blood 87: 2621-2631.
2. Stewart, D.M., et al. 1996. Studies of the expression of the Wiskott-Aldrich syndrome protein. J. Clin. Invest. 97: 2627-2634.
3. Symons, M., et al. 1996. Wiskott-Aldrich syndrome protein, a novel effector for the GTPase CDC42Hs, is implicated in Actin polymerization. Cell 84: 723-734.
4. Miki, H., et al. 1996. N-WASP, a novel actin-depolymerizing protein, regulates the cortical cytoskeletal rearrangement in a PIP2-dependent manner downstream of tyrosine kinases. EMBO J. 15: 5326-5335.
5. Miki, H., et al. 1998. Direct binding of the verprolin-homology domain in N-WASP to Actin is essential for cytoskeletal reorganization. Biochem. Biophys. Res. Commun. 243: 73-78.
6. Miki, H., et al. 1998. Induction of filopodium formation by a WASP-related Actin-depolymerizing protein N-WASP. Nature 391: 93-96.

CHROMOSOMAL LOCATION

Genetic locus: Wasl (mouse) mapping to 6 A3.1.

PRODUCT

N-WASP siRNA (m) is a pool of 3 target-specific 19-25 nt siRNAs designed to knock down gene expression. Each vial contains 3.3 nmol of lyophilized siRNA, sufficient for a 10 µM solution once resuspended using protocol below. Suitable for 50-100 transfections. Also see N-WASP shRNA Plasmid (m): sc-36007-SH and N-WASP shRNA (m) Lentiviral Particles: sc-36007-V as alternate gene silencing products.

For independent verification of N-WASP (m) gene silencing results, we also provide the individual siRNA duplex components. Each is available as 3.3 nmol of lyophilized siRNA. These include: sc-36007A, sc-36007B and sc-36007C.

STORAGE AND RESUSPENSION

Store lyophilized siRNA duplex at -20° C with desiccant. Stable for at least one year from the date of shipment. Once resuspended, store at -20° C, avoid contact with RNAses and repeated freeze thaw cycles.

Resuspend lyophilized siRNA duplex in 330 µl of the RNAse-free water provided. Resuspension of the siRNA duplex in 330 µl of RNAse-free water makes a 10 µM solution in a 10 µM Tris-HCl, pH 8.0, 20 mM NaCl, 1 mM EDTA buffered solution.

APPLICATIONS

N-WASP siRNA (m) is recommended for the inhibition of N-WASP expression in mouse cells.

SUPPORT REAGENTS

For optimal siRNA transfection efficiency, Santa Cruz Biotechnology's siRNA Transfection Reagent: sc-29528 (0.3 ml), siRNA Transfection Medium: sc-36868 (20 ml) and siRNA Dilution Buffer: sc-29527 (1.5 ml) are recommended. Control siRNAs or Fluorescein Conjugated Control siRNAs are available as 10 µM in 66 µl. Each contain a scrambled sequence that will not lead to the specific degradation of any known cellular mRNA. Fluorescein Conjugated Control siRNAs include: sc-36869, sc-44239, sc-44240 and sc-44241. Control siRNAs include: sc-37007, sc-44230, sc-44231, sc-44232, sc-44233, sc-44234, sc-44235, sc-44236, sc-44237 and sc-44238.

GENE EXPRESSION MONITORING

N-WASP (C-1): sc-271484 is recommended as a control antibody for monitoring of N-WASP gene expression knockdown by Western Blotting (starting dilution 1:200, dilution range 1:100-1:1000) or immunofluorescence (starting dilution 1:50, dilution range 1:50-1:500).

To ensure optimal results, the following support reagents are recommended: 1) Western Blotting: use m-IgGκ BP-HRP: sc-516102 or m-IgGκ BP-HRP (Cruz Marker): sc-516102-CM (dilution range: 1:1000-1:10000), Cruz Marker™ Molecular Weight Standards: sc-2035, UltraCruz® Blocking Reagent: sc-516214 and Western Blotting Luminol Reagent: sc-2048. 2) Immunofluorescence: use m-IgGκ BP-FITC: sc-516140 or m-IgGκ BP-PE: sc-516141 (dilution range: 1:50-1:200) with UltraCruz® Mounting Medium: sc-24941 or UltraCruz® Hard-set Mounting Medium: sc-359850.

RT-PCR REAGENTS

Semi-quantitative RT-PCR may be performed to monitor N-WASP gene expression knockdown using RT-PCR Primer: N-WASP (m)-PR: sc-36007-PR (20 µl, 470 bp). Annealing temperature for the primers should be 55-60° C and the extension temperature should be 68-72° C.

RESEARCH USE

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

PROTOCOLS

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