WASP siRNA (h): sc-29525



The Power to Question

BACKGROUND

The Wiskott-Aldrich syndrome (WAS) is a disorder that results from a monogenic defect that has been mapped to the short arm of the X chromosome. WAS is characterized by thrombocytopenia, eczema, defects in cell-mediated and humoral immunity and a propensity for lymphoproliferative disease. The gene that is mutated in the syndrome encodes a proline-rich protein of unknown function designated WAS protein (WASP). A clue to WASP function came from the observation that T cells from affected males had an irregular cellular morphology and a disarrayed cytoskeleton suggesting the involvement of WASP in cytoskeletal organization. Close examination of the WASP sequence revealed a putative Cdc42/Rac interacting domain, homologous with those found in PAK65 and ACK. Subsequent investigation has shown WASP to be a true downstream effector of Cdc42.

REFERENCES

- 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.
- 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.
- Kolluri, R., et al. 1996. Direct interaction of the Wiskott-Aldrich syndrome protein with the GTPase Cdc42. Proc. Natl. Acad. Sci. USA 93: 5615-5618.
- Schindelhauer, D., et al. 1996. Wiskott-Aldrich syndrome: no strict genotype-phenotype correlations but clustering of missense mutations in the amino-terminal part of the WASP gene product. Hum. Genet. 98: 68-76.

CHROMOSOMAL LOCATION

Genetic locus: WAS (human) mapping to Xp11.23.

PRODUCT

WASP siRNA (h) 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 WASP shRNA Plasmid (h): sc-29525-SH and WASP shRNA (h) Lentiviral Particles: sc-29525-V as alternate gene silencing products.

For independent verification of WASP (h) gene silencing results, we also provide the individual siRNA duplex components. Each is available as 3.3 nmol of lyophilized siRNA. These include: sc-29525A, sc-29525B and sc-29525C.

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.

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

APPLICATIONS

WASP siRNA (h) is recommended for the inhibition of WASP expression in human 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

WASP (D-1): sc-5300 is recommended as a control antibody for monitoring of 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-lgG κ BP-HRP: sc-516102 or m-lgG κ BP-HRP (Cruz Marker): sc-516102-CM (dilution range: 1:1000-1:10000), Cruz MarkerTM Molecular Weight Standards: sc-2035, UltraCruz[®] Blocking Reagent: sc-516214 and Western Blotting Luminol Reagent: sc-2048. 2) Immunofluorescence: use m-lgG κ BP-FITC: sc-516140 or m-lgG κ 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 WASP gene expression knockdown using RT-PCR Primer: WASP (h)-PR: sc-29525-PR (20 μ l, 569 bp). Annealing temperature for the primers should be 55-60° C and the extension temperature should be 68-72° C.

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