SANTA CRUZ BIOTECHNOLOGY, INC.

WRP siRNA (h): sc-76929

BACKGROUND

WAVE-associated Rac GTPase-activating protein (WRP), also known as SLIT-ROBO Rho GTPase-activating protein 3 (srGAP3) and Mental disorder-activating GAP (MEGAP), is a 1,099 amino acid protein containing one FCH domain, one Rho-GAP domain and one SH3 domain. Expressed highly in brain, and in lower levels in kidney, WRP is thought to play a role in cell migration through its interaction with Cdc42 and Rac1. Cdc42 and Rac1 are two intracellular signaling proteins that regulate the multistep cell migration process. WRP downregulates Cdc42 and Rac1 activity, thereby impairing Actin and microtubule dynamics, the formation of protrusions, and total cell migration. Defects in the gene encoding WRP have been linked to severe idiopathic mental retardation. Three isoforms of WRP exist as a result of alternative splicing events.

REFERENCES

1. Miki, H., et al. 1998. WAVE, a novel WASP-family protein involved in Actin reorganization induced by Rac. EMBO J. 17: 6932-6941.
2. Wong, K., et al. 2001. Signal transduction in neuronal migration: roles of GTPase activating proteins and the small GTPase Cdc42 in the Slit-Robo pathway. Cell 107: 209-221.
3. Soderling, S.H., et al. 2002. The WRP component of the WAVE-1 complex attenuates Rac-mediated signalling. Nat. Cell Biol. 4: 970-975.
4. Endris, V., et al. 2002. The novel Rho-GTPase activating gene MEGAP/srGAP3 has a putative role in severe mental retardation. Proc. Natl. Acad. Sci. USA 99: 11754-11759.
5. Miki, H. and Takenawa, T. 2003. Regulation of actin dynamics by WASP family proteins. J. Biochem. 134: 309-313.
6. Soderling, S.H., et al. 2007. A WAVE-1 and WRP signaling complex regulates spine density, synaptic plasticity, and memory. J. Neurosci. 27: 355-365.
7. Waltereit, R., et al. 2008. Expression of MEGAP mRNA during embryonic development. Gene Expr. Patterns 8: 307-310.
8. Bacon, C., et al. 2009. Dynamic expression of the Slit-Robo GTPase activating protein genes during development of the murine nervous system. J. Comp. Neurol. 513: 224-236.

CHROMOSOMAL LOCATION

Genetic locus: SRGAP3 (human) mapping to 3p25.3.

PRODUCT

WRP 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 WRP shRNA Plasmid (h): sc-76929-SH and WRP shRNA (h) Lentiviral Particles: sc-76929-V as alternate gene silencing products.

For independent verification of WRP (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-76929A, sc-76929B and sc-76929C.

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

WRP siRNA (h) is recommended for the inhibition of WRP 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

WRP (E-11): sc-374503 is recommended as a control antibody for monitoring of WRP 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 WRP gene expression knockdown using RT-PCR Primer: WRP (h)-PR: sc-76929-PR (20 µl). 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.