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# WAVE3 shRNA (h) Lentiviral Particles: sc-44192-V

## BACKGROUND

WASP (for Wiskott-Aldrich syndrome protein) and N-WASP are downstream effectors of Cdc42 that are implicated in Actin polymerization and cytoskeletal organization. The WASP family also includes VASP (vasodilator-stimulated phosphoprotein) and Mena (for mammalian enabled protein), which accumulate at focal adhesions and are also involved in the regulation of the Actin cytoskeleton. The WAVE proteins are related to the WASP family proteins and are likewise involved in mediating Actin reorganization downstream of the Rho family of small GTPases. The two protein homologs WAVE1 and WAVE2 specifically regulate membrane ruffling by inducing the formation of Actin filament clusters in response to GTP binding and activating Rac. The WAVE proteins mediate this Actin polymerization by cooperating with the Arp2/3 complex, a nucleation core, and thereby promoting the formation of Actin filaments. WAVE1, which is also designated SCAR (for suppressor of cAR), is expressed primarily in the brain, while WAVE2 is widely expressed with the expression highest in peripheral blood leukocytes.

## REFERENCES

1. 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.
2. Miki, H., et al. 1998. WAVE, a novel WASP-family protein involved in Actin reorganization induced by Rac. *EMBO J.* 17: 6932-6941.
3. Machesky, L.M. and Insall, R.H. 1998. Scar1 and the related Wiskott-Aldrich syndrome protein, WASP, regulate the Actin cytoskeleton through the Arp2/3 complex. *Curr. Biol.* 8: 1347-1356.
4. Bear, J.E., et al. 1998. SCAR, a WASP-related protein, isolated as a suppressor of receptor defects in late *Dictyostelium* development. *J. Cell Biol.* 142: 1325-1335.
5. Rohatgi, R., et al. 1999. The interaction between N-WASP and the Arp2/3 complex links Cdc42-dependent signals to actin assembly. *Cell* 97: 221-231.

## CHROMOSOMAL LOCATION

Genetic locus: WASF3 (human) mapping to 13q12.13.

## PRODUCT

WAVE3 shRNA (h) Lentiviral Particles is a pool of concentrated, transduction-ready viral particles containing 3 target-specific constructs that encode 19-25 nt (plus hairpin) shRNA designed to knock down gene expression. Each vial contains 200  $\mu$ l frozen stock containing  $1.0 \times 10^6$  infectious units of virus (IFU) in Dulbecco's Modified Eagle's Medium with 25 mM HEPES pH 7.3. Suitable for 10-20 transductions. Also see WAVE3 siRNA (h): sc-44192 and WAVE3 shRNA Plasmid (h): sc-44192-SH as alternate gene silencing products.

## STORAGE

Store lentiviral particles at  $-80^{\circ}$  C. Stable for at least one year from the date of shipment. Once thawed, particles can be stored at  $4^{\circ}$  C for up to one week. Avoid repeated freeze thaw cycles.

## APPLICATIONS

WAVE3 shRNA (h) Lentiviral Particles is recommended for the inhibition of WAVE3 expression in human cells.

## SUPPORT REAGENTS

Control shRNA Lentiviral Particles: sc-108080. Available as 200  $\mu$ l frozen viral stock containing  $1.0 \times 10^6$  infectious units of virus (IFU); contains an shRNA construct encoding a scrambled sequence that will not lead to the specific degradation of any known cellular mRNA.

## GENE EXPRESSION MONITORING

WAVE3 (E-3): sc-515303 is recommended as a control antibody for monitoring of WAVE3 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 (secondary) reagents are recommended: 1) Western Blotting: use goat anti-mouse IgM-HRP: sc-2064 (dilution range: 1:500-1:5,000), TBS Blotto A Blocking Reagent: sc-2333 and Western Blotting Luminol Reagent: sc-2048. 2) Immunofluorescence: use goat anti-mouse IgM-FITC: sc-2082 (dilution range: 1:100-1:400) or goat anti-mouse IgM-TR: sc-2983 (dilution range: 1:100-1:400) with UltraCruz<sup>™</sup> Mounting Medium: sc-24941.

## RT-PCR REAGENTS

Semi-quantitative RT-PCR may be performed to monitor WAVE3 gene expression knockdown using RT-PCR Primer: WAVE3 (h)-PR: sc-44192-PR (20  $\mu$ l). Annealing temperature for the primers should be  $55-60^{\circ}$  C and the extension temperature should be  $68-72^{\circ}$  C.

## BIOSAFETY

Lentiviral particles can be employed in standard Biosafety Level 2 tissue culture facilities (and should be treated with the same level of caution as with any other potentially infectious reagent). Lentiviral particles are replication-incompetent and are designed to self-inactivate after transduction and integration of shRNA constructs into genomic DNA of target cells.

## RESEARCH USE

The purchase of this product conveys to the buyer the nontransferable right to use the purchased amount of the product and all replicates and derivatives for research purposes conducted by the buyer in his laboratory only (whether the buyer is an academic or for-profit entity). The buyer cannot sell or otherwise transfer (a) this product (b) its components or (c) materials made using this product or its components to a third party, or otherwise use this product or its components or materials made using this product or its components for Commercial Purposes.

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