

DAAM1 siRNA (m): sc-62191

BACKGROUND

DAAM1 (dishevelled associated activator of morphogenesis 1) is a 1,078 amino acid member of the formin homology protein family. Localized to the perinuclear cytoplasm and expressed throughout the body, DAAM1 binds to dishevelled (Dvl) and Rho and mediates the Wnt-induced formation of the Dvl-Rho complex. Once complexed to Dvl, Rho becomes activated and can regulate cell polarity, movement and cytoskeletal architecture. Activation of Rho is dependent upon formation of the Dvl-Rho complex. This suggests that DAAM1 (which is required for complex formation) is a critical component of cellular cortex functions. DAAM1 contains several binding domains which allow it to interact with various proteins such as CIP4, FNB1 and spectrin, thereby helping to coordinate the dynamics of the Actin filament system. Additionally, DAAM1 is thought to act as a scaffolding protein by recruiting Rho-GEF and Rho-GDP, thus enhancing Rho-GTP formation. Three distinct isoforms exist due to alternative splicing events.

REFERENCES

1. Habas, R., et al. 2002. Wnt/Frizzled activation of Rho regulates vertebrate gastrulation and requires a novel formin homology protein DAAM1. *Cell* 107: 843-854.
2. Kida, Y., et al. 2004. Identification of chick and mouse DAAM1 and DAAM2 genes and their expression patterns in the central nervous system. *Brain Res. Dev. Brain Res.* 153: 143-150.
3. Nakaya, M.A., et al. 2004. Identification and comparative expression analyses of DAAM genes in mouse and *Xenopus*. *Gene Expr. Patterns* 5: 97-105.

CHROMOSOMAL LOCATION

Genetic locus: Daam1 (mouse) mapping to 12 C3.

PRODUCT

DAAM1 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 μ M solution once resuspended using protocol below. Suitable for 50-100 transfections. Also see DAAM1 shRNA Plasmid (m): sc-62191-SH and DAAM1 shRNA (m) Lentiviral Particles: sc-62191-V as alternate gene silencing products.

For independent verification of DAAM1 (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-62191A, sc-62191B and sc-62191C.

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

DAAM1 siRNA (m) is recommended for the inhibition of DAAM1 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 μ M in 66 μ l. 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

DAAM1 (WW-3): sc-100942 is recommended as a control antibody for monitoring of DAAM1 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-IgG κ BP-HRP: sc-516102 or m-IgG κ 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-IgG κ BP-FITC: sc-516140 or m-IgG 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 DAAM1 gene expression knockdown using RT-PCR Primer: DAAM1 (m)-PR: sc-62191-PR (20 μ l, 419 bp). Annealing temperature for the primers should be 55-60° C and the extension temperature should be 68-72° C.

SELECT PRODUCT CITATIONS

1. Suzuki, J.I., et al. 2017. Depletion of tumor suppressor Kank1 induces centrosomal amplification via hyperactivation of RhoA. *Exp. Cell Res.* 353: 79-87.

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.