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AF-6 siRNA (h): sc-43007

BACKGROUND

The dynamics of a cell-cell interface such as tight junctions or adherens junctions are important in many developmental, physiological and pathological processes. AF-6 (MLLT4: myeloid/lymphoid or mixed-lineage leukemia translocated to 4) is a 1,612 amino acid protein that contains two N-terminal Ras binding domains (RBD) and a GLGF motif, and is implicated in Ras-mediated signaling events occurring at peripheral cell-cell junctions. AF-6 interacts with F-Actin and Profilin in cell-cell junctions, and may modulate Actin modeling near adhesion complexes. Furthermore, AF-6 coordinates junction adhesion molecule (JAM) recruitment to intercellular junctions through a PDZ domain. Developing mice deficient in AF-6 activity display a loss of neuroepithelial polarity, suggesting that AF-6 activity is an important regulator of cell-cell junctions that influence apical/basolateral asymmetry.

REFERENCES

1. Prasad, R., et al. 1993. Cloning of the ALL-1 fusion partner, the AF-6 gene, involved in acute myeloid leukemias with the t(6;11) chromosome translocation. *Cancer Res.* 53: 5624-5628.
2. Kuriyama, M., et al. 1996. Identification of AF-6 and canoe as putative targets for Ras. *J. Biol. Chem.* 271: 607-610.
3. Yamamoto, T., et al. 1997. The Ras target AF-6 interacts with ZO-1 and serves as a peripheral component of tight junctions in epithelial cells. *J. Cell Biol.* 139: 785-795.
4. Zhadanov, A.B., et al. 1999. Absence of the tight junctional protein AF-6 disrupts epithelial cell-cell junctions and cell polarity during mouse development. *Curr. Biol.* 9: 880-888.

CHROMOSOMAL LOCATION

Genetic locus: MLLT4 (human) mapping to 6q27.

PRODUCT

AF-6 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 μ M solution once resuspended using protocol below. Suitable for 50-100 transfections. Also see AF-6 shRNA Plasmid (h): sc-43007-SH and AF-6 shRNA (h) Lentiviral Particles: sc-43007-V as alternate gene silencing products.

For independent verification of AF-6 (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-43007A, sc-43007B and sc-43007C.

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

AF-6 siRNA (h) is recommended for the inhibition of AF-6 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 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

AF-6 (B-5): sc-74433 is recommended as a control antibody for monitoring of AF-6 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 AF-6 gene expression knockdown using RT-PCR Primer: AF-6 (h)-PR: sc-43007-PR (20 μ l, 459 bp). Annealing temperature for the primers should be 55-60° C and the extension temperature should be 68-72° C.

SELECT PRODUCT CITATIONS

1. Marques, M.S., et al. 2018. Afadin downregulation by *Helicobacter pylori* induces epithelial to mesenchymal transition in gastric cells. *Front. Microbiol.* 9: 2712.

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.