SANTA CRUZ BIOTECHNOLOGY, INC.

AIP4 siRNA (h, m): sc-40364

BACKGROUND

Atrophin interacting proteins (AIP)s bind to atrophin-1 in the vicinity of the polyglutamine tract. The WW domain consists of 35-40 amino acids and is characterized by four well conserved aromatic residues, two of which are tryptophan. All five AIPs contain multiple WW domains and can be divided into two distinct classes. AIP1 and AIP3 (WWP3) are MAGUK-like multidomain proteins containing a guanylate kinase-like region, two WW domains, and multiple PDZ domains. AIP2 (WWP2), AIP4 (itchy), and AIP5 (WWP1) are highly homologous, each having four WW domains and a HECT domain characteristic of ubiquitin ligases. These interactors are similar to isolated Huntingtin-interacting proteins, suggesting commonality of function between two families of proteins responsible for similar diseases.

CHROMOSOMAL LOCATION

Genetic locus: ITCH (human) mapping to 20q11.22; Itch (mouse) mapping to 2 H1.

PRODUCT

AIP4 siRNA (h, m) is a target-specific 19-25 nt siRNA 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 AIP4 shRNA Plasmid (h, m): sc-40364-SH and AIP4 shRNA (h, m) Lentiviral Particles: sc-40364-V as alternate gene silencing 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 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

AIP4 siRNA (h, m) is recommended for the inhibition of AIP4 expression in human and 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 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.

RESEARCH USE

For research use only, not for use in diagnostic procedures.

GENE EXPRESSION MONITORING

AIP4 (G-11): sc-28367 is recommended as a control antibody for monitoring of AIP4 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 AIP4 gene expression knockdown using RT-PCR Primer: AIP4 (h, m)-PR: sc-40364-PR (20 µl, 500 bp). Annealing temperature for the primers should be 55-60° C and the extension temperature should be 68-72° C.

SELECT PRODUCT CITATIONS

1. Moreno-García, M.E., et al. 2013. Kinase-independent feedback of the TAK1/TAB1 complex on Bcl10 turnover and NFκB activation. Mol. Cell. Biol. 33: 1149-1163.
2. Shukla, S., et al. 2014. KRAS protein stability is regulated through Smurf2: UBCH5 complex-mediated β-TrCP1 degradation. Neoplasia 16: 115-128.
3. Park, S.H., et al. 2015. Itch E3 ubiquitin ligase positively regulates TGF-β signaling to EMT via Smad7 ubiquitination. Mol. Cells 38: 20-25.
4. Park, J.Y., et al. 2016. cAMP signaling increases histone deacetylase 8 expression by inhibiting JNK-dependent degradation via autophagy and the proteasome system in H1299 lung cancer cells. Biochem. Biophys. Res. Commun. 470: 336-342.
5. Yang, C., et al. 2018. A20/TNFAIP3 regulates the DNA damage response and mediates tumor cell resistance to DNA-damaging therapy. Cancer Res. 78: 1069-1082.
6. Park, S.H., et al. 2019. Codium fragile F2 sensitize colorectal cancer cells to TRAIL-induced apoptosis via c-FLIP ubiquitination. Biochem. Biophys. Res. Commun. 508: 1-8.
7. Ruan, T., et al. 2022. H5N1 infection impairs the alveolar epithelial barrier through intercellular junction proteins via Itch-mediated proteasomal degradation. Commun. Biol. 5: 186.

PROTOCOLS

See our web site at www.scbt.com for detailed protocols and support products.