

AIP5 siRNA (m): sc-40367

BACKGROUND

Atrophin interacting proteins (AIPs) 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.

REFERENCES

1. Bork, P. and Sudol, M. 1994. The WW domain: a signalling site in dystrophin? *Trends Biochem. Sci.* 19: 531-533.
2. Andre, B. and Springael, J.Y. 1994. WWP, a new amino acid motif present in single or multiple copies in various proteins including dystrophin and the SH3-binding Yes-associated protein YAP65. *Biochem. Biophys. Res. Commun.* 205: 1201-1205.
3. Hofmann, K. and Bucher, P. 1995. The Rsp5-domain is shared by proteins of diverse functions. *FEBS Lett.* 358: 153-157.
4. Pirozzi, G., et al. 1997. Identification of novel human WW domain-containing proteins by cloning of ligand targets. *J. Biol. Chem.* 272: 14611-14616.
5. Perry, W.L., et al. 1998. The itchy locus encodes a novel ubiquitin protein ligase that is disrupted in a^{18H} mice. *Nat. Genet.* 18: 143-146.

CHROMOSOMAL LOCATION

Genetic locus: Wwp1 (mouse) mapping to 4 A3.

PRODUCT

AIP5 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 AIP5 shRNA Plasmid (m): sc-40367-SH and AIP5 shRNA (m) Lentiviral Particles: sc-40367-V as alternate gene silencing products.

For independent verification of AIP5 (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-40367A, sc-40367B and sc-40367C.

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

AIP5 siRNA (m) is recommended for the inhibition of AIP5 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

AIP5 (F-3): sc-390897 is recommended as a control antibody for monitoring of AIP5 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 AIP5 gene expression knockdown using RT-PCR Primer: AIP5 (m)-PR: sc-40367-PR (20 μ l). Annealing temperature for the primers should be 55-60° C and the extension temperature should be 68-72° C.

SELECT PRODUCT CITATIONS

1. Zhao, L., et al. 2011. Tumor necrosis factor inhibits mesenchymal stem cell differentiation into osteoblasts via the ubiquitin E3 ligase Wwp1. *Stem Cells* 29: 1601-1610.

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.