

WIPI-4 siRNA (h): sc-72216

BACKGROUND

WD-repeats are motifs that are found in a variety of proteins and are characterized by a conserved core of 40-60 amino acids that commonly form a tertiary propeller structure. While proteins that contain WD-repeats participate in a wide range of cellular functions, they are generally involved in regulatory mechanisms concerning chromatin assembly, cell cycle control, signal transduction, RNA processing, apoptosis and vesicular trafficking. WIPI-4 (WD repeat domain phosphoinositide-interacting protein 4), also known as WDR45 (WD repeat domain 45), JM5 or WDRX1, is a 360 amino acid protein containing three WD repeats. Existing as three alternatively spliced isoforms, WIPI-4 is ubiquitously expressed but found at highest levels in heart and skeletal muscle.

REFERENCES

1. Clark, A.G., et al. 2003. Inferring nonneutral evolution from human-chimp-mouse orthologous gene trios. *Science* 302: 1960-1963.
2. Jeffries, T.R., et al. 2004. PtdIns-specific MPR pathway association of a novel WD40 repeat protein, WIPI49. *Mol. Biol. Cell* 15: 2652-2663.
3. Proikas-Cezanne, T., et al. 2004. WIPI-1 α (WIPI49), a member of the novel 7-bladed WIPI protein family, is aberrantly expressed in human cancer and is linked to starvation-induced autophagy. *Oncogene* 23: 9314-9325.
4. Online Mendelian Inheritance in Man, OMIM™. 2005. Johns Hopkins University, Baltimore, MD. MIM Number: 300526. World Wide Web URL: <http://www.ncbi.nlm.nih.gov/omim/>
5. Proikas-Cezanne, T., et al. 2007. Human WIPI-1 puncta-formation: a novel assay to assess mammalian autophagy. *FEBS Lett.* 581: 3396-3404.
6. Hudson, A.M. and Cooley, L. 2008. Phylogenetic, structural and functional relationships between WD- and Kelch-repeat proteins. *Subcell. Biochem.* 48: 6-19.

CHROMOSOMAL LOCATION

Genetic locus: WDR45 (human) mapping to Xp11.23.

PRODUCT

WIPI-4 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 WIPI-4 shRNA Plasmid (h): sc-72216-SH and WIPI-4 shRNA (h) Lentiviral Particles: sc-72216-V as alternate gene silencing products.

For independent verification of WIPI-4 (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-72216A, sc-72216B and sc-72216C.

PROTOCOLS

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

WIPI-4 siRNA (h) is recommended for the inhibition of WIPI-4 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

WIPI-4 (G-12): sc-398272 is recommended as a control antibody for monitoring of WIPI-4 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 WIPI-4 gene expression knockdown using RT-PCR Primer: WIPI-4 (h)-PR: sc-72216-PR (20 μ l). Annealing temperature for the primers should be 55-60° C and the extension temperature should be 68-72° C.

RESEARCH USE

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