SANTA CRUZ BIOTECHNOLOGY, INC.

WIPI-3 siRNA (h): sc-72214

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-3 (WD repeat domain phosphoinositide-interacting protein 3), also known as WD repeat-containing protein 45-like or WIPI49-like protein, is a 344 amino acid protein that is ubiquitously expressed with highest levels found in pancreas, heart and skeletal muscle. Upregulated in uterine and ovarian cancer, WIPI-3 contains two WD domains, seven WD40 domains, and is encoded by a gene that maps to human chromosome 17q25.3. Chromosome 17 comprises over 2.5% of the human genome, encodes over 1,200 genes and is associated with two key tumor suppressor genes, namely, p53 and BRCA1.

REFERENCES

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3. Minamoto, T., et al. 2001. Distinct pattern of p53 phosphorylation in human tumors. Oncogene 20: 3341-3347.
4. Jeffries, T.R., et al. 2004. PtdIns-specific MPR pathway association of a novel WD40 repeat protein, WIPI49. Mol. Biol. Cell 15: 2652-2663.
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7. Proikas-Cezanne, T., et al. 2007. Human WIPI-1 puncta-formation: a novel assay to assess mammalian autophagy. FEBS Lett. 58: 3396-3404.

CHROMOSOMAL LOCATION

Genetic locus: WDR45L (human) mapping to 17q25.3.

PRODUCT

WIPI-3 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 µM solution once resuspended using protocol below. Suitable for 50-100 transfections. Also see WIPI-3 shRNA Plasmid (h): sc-72214-SH and WIPI-3 shRNA (h) Lentiviral Particles: sc-72214-V as alternate gene silencing products.

For independent verification of WIPI-3 (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-72214A, sc-72214B and sc-72214C.

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

ARMER siRNA (m) is recommended for the inhibition of ARMER 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 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.

GENE EXPRESSION MONITORING

WIPI-3 (B-7): sc-514194 is recommended as a control antibody for monitoring of WIPI-3 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 WIPI-3 gene expression knockdown using RT-PCR Primer: WIPI-3 (h)-PR: sc-72214-PR (20 µl). 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.

PROTOCOLS

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