SANTA CRUZ BIOTECHNOLOGY, INC.

WDFY3 siRNA (h): sc-89191

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. WDFY3 (WD repeat and FYVE domain containing 3), also known as ALFY (autophagy-linked FYVE protein) is a 3,526 amino acid protein that localizes to the cytoplasmic side of peripheral membranes. Ubiquitously expressed, WDFY3 co-localizes with autophagic structures in starved cells and is expressed in liver, brain and kidney. WDFY3 exists as two alternatively spliced isoforms and contains one BEACH domain, one FYVE-type zinc finger, a pair of LRR (leucine-rich) repeats and five WD repeats.

REFERENCES

1. Gaullier, J.M., et al. 1998. FYVE fingers bind PtdIns(3)P. Nature 394: 432-433.
2. Gillooly, D.J., et al. 2001. Cellular functions of phosphatidylinositol 3-phosphate and FYVE domain proteins. Biochem. J. 355: 249-258.
3. Chen, G.Y., et al. 2004. Expression profile of mouse BWF1, a protein with a BEACH domain, WD40 domain and FYVE domain. Cell Struct. Funct. 29: 35-42.
4. Hayakawa, A., et al. 2004. Structural basis for endosomal targeting by FYVE domains. J. Biol. Chem. 279: 5958-5966.
5. Simonsen, A., et al. 2004. Alfy, a novel FYVE-domain-containing protein associated with protein granules and autophagic membranes. J. Cell Sci. 117: 4239-4251.
6. Wang, Y., et al. 2006. Tyrosine phosphorylated PAR-3 regulates epithelial tight junction assembly promoted by EGFR signaling. EMBO J. 25: 5058-5070.
7. Hayakawa, A., et al. 2006. The WD40 and FYVE domain containing protein 2 defines a class of early endosomes necessary for endocytosis. Proc. Natl. Acad. Sci. USA 103: 11928-11933.

CHROMOSOMAL LOCATION

Genetic locus: WDFY3 (human) mapping to 4q21.23.

PRODUCT

WDFY3 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 WDFY3 shRNA Plasmid (h): sc-89191-SH and WDFY3 shRNA (h) Lentiviral Particles: sc-89191-V as alternate gene silencing products.

For independent verification of WDFY3 (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-89191A, sc-89191B and sc-89191C.

RESEARCH USE

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

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

WDFY3 siRNA (h) is recommended for the inhibition of WDFY3 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 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

WDFY3 (B-4): sc-514569 is recommended as a control antibody for monitoring of WDFY3 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 WDFY3 gene expression knockdown using RT-PCR Primer: WDFY3 (h)-PR: sc-89191-PR (20 µl). Annealing temperature for the primers should be 55-60° C and the extension temperature should be 68-72° C.

PROTOCOLS

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