

14-3-3 σ siRNA (m): sc-29591

BACKGROUND

14-3-3 proteins regulate many cellular processes relevant to cancer biology, notably apoptosis, mitogenic signaling and cell-cycle checkpoints. Seven isoforms, denoted 14-3-3 β , γ , ϵ , ζ , η , θ and σ , comprise this family of signaling intermediates. 14-3-3 σ , also known as SFN, stratifin, HME1 or YWHAS, is a secreted adaptor protein that is involved in regulating both general and specific signaling pathways. Expressed predominately in stratified squamous keratinising epithelium, 14-3-3 σ is able to bind and modify the activity of a large number of proteins, such as KRT17 (Keratin 17), through recognition of a phosphothreonine or phosphoserine motif. When bound to Keratin 17, for example, 14-3-3 σ acts to stimulate the Akt/mTOR signaling pathway by upregulating protein synthesis and cell growth. 14-3-3 σ also functions to positively mediate IGF-I-induced cell cycle progression and can bind to a variety of translation initiation factors, thus controlling mitotic translation. In response to tumor growth, 14-3-3 σ positively regulates the tumor suppressor p53 and increases the rate of p53-regulated inhibition of G₂/M cell cycle progression. Multiple isoforms of 14-3-3 σ exist due to alternative splicing events.

REFERENCES

1. Yang, H.Y., et al. 2003. 14-3-3 σ positively regulates p53 and suppresses tumor growth. *Mol. Cell. Biol.* 23: 7096-7107.
2. Wilker, E.W., et al. 2005. A structural basis for 14-3-3 σ functional specificity. *J. Biol. Chem.* 280: 18891-18898.
3. Pulukuri, S.M. and Rao, J.S. 2006. CpG island promoter methylation and silencing of 14-3-3 σ gene expression in LNCaP and Tramp-C1 prostate cancer cell lines is associated with methyl-CpG-binding protein MBD2. *Oncogene* 25: 4559-4572.

CHROMOSOMAL LOCATION

Genetic locus: Sfn (mouse) mapping to 4 D2.3.

PRODUCT

14-3-3 σ siRNA (m) is a pool of 2 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 14-3-3 σ shRNA Plasmid (m): sc-29591-SH and 14-3-3 σ shRNA (m) Lentiviral Particles: sc-29591-V as alternate gene silencing products.

For independent verification of 14-3-3 σ (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-29591A and sc-29591B.

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

14-3-3 σ siRNA (m) is recommended for the inhibition of 14-3-3 σ 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

14-3-3 σ (E-11): sc-166473 is recommended as a control antibody for monitoring of 14-3-3 σ 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 14-3-3 σ gene expression knockdown using RT-PCR Primer: 14-3-3 σ (m)-PR: sc-29591-PR (20 μ l, 452 bp). Annealing temperature for the primers should be 55-60° C and the extension temperature should be 68-72° C.

SELECT PRODUCT CITATIONS

1. Liou, J.Y., et al. 2017. An efficient transfection method for differentiation and cell proliferation of mouse embryonic stem cells. *Methods Mol. Biol.* 1622: 139-147.
2. Kim, M., et al. 2017. UVB-induced nuclear translocation of TC-PTP by AKT/14-3-3 σ axis inhibits keratinocyte survival and proliferation. *Oncotarget* 8: 90674-90692.

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.