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14-3-3 ζ siRNA (r): sc-156019

BACKGROUND

14-3-3 proteins regulate many cellular processes relevant to cancer biology, notably apoptosis, mitogenic signaling and cell-cycle checkpoints. Seven isoforms comprise this family of signaling intermediates, denoted 14-3-3 β , γ , ϵ , ζ , η , θ and σ . 14-3-3 proteins form dimers that present two binding sites for ligand proteins, thereby bringing together two proteins that may not otherwise associate. These ligands largely share a 14-3-3 consensus binding motif and exhibit serine/threonine phosphorylation. 14-3-3 proteins function in broad regulation of these ligand proteins, by cytoplasmic sequestration, occupation of interaction domains and import/export sequences, prevention of degradation, activation/repression of enzymatic activity and facilitation of protein modification, and thus loss of expression contributes to a vast array of pathogenic cellular activities.

REFERENCES

- Morrison, D. 1994. 14-3-3: modulators of signaling proteins? *Science* 266: 56-57.
- Muratake, T., et al. 1996. Structural organization and chromosomal assignment of the human 14-3-3 β chain gene (YWHAH). *Genomics* 36: 63-69.
- Yaffe, M.B., et al. 1997. The structural basis for 14-3-3 phosphopeptide binding specificity. *Cell* 91: 961-971.
- Megidish, T., et al. 1998. A novel sphingosine-dependent protein kinase (SDK1) specifically phosphorylates certain isoforms of 14-3-3 protein. *J. Biol. Chem.* 273: 21834-21845.

CHROMOSOMAL LOCATION

Genetic locus: Ywhaz (rat) mapping to 7q22.

PRODUCT

14-3-3 ζ siRNA (r) 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 14-3-3 ζ shRNA Plasmid (r): sc-156019-SH and 14-3-3 ζ shRNA (r) Lentiviral Particles: sc-156019-V as alternate gene silencing products.

For independent verification of 14-3-3 ζ (r) gene silencing results, we also provide the individual siRNA duplex components. Each is available as 3.3 nmol of lyophilized siRNA. These include: sc-156019A, sc-156019B and sc-156019C.

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 ζ siRNA (r) is recommended for the inhibition of 14-3-3 ζ expression in rat 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 ζ (1B3): sc-293415 is recommended as a control antibody for monitoring of 14-3-3 ζ 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 ζ gene expression knockdown using RT-PCR Primer: 14-3-3 ζ (r)-PR: sc-156019-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

- He, Y., et al. 2010. LC-MS/MS analysis of ovarian cancer metastasis-related proteins using a nude mouse model: 14-3-3 ζ as a candidate biomarker. *J. Proteome Res.* 9: 6180-6190.

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.