

# 14-3-3 $\gamma$ siRNA (h): sc-29582

## 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  $\beta$ ,  $\gamma$ ,  $\epsilon$ ,  $\zeta$ ,  $\eta$ ,  $\theta$  and  $\sigma$ . 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  $\beta$  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: YWHAG (human) mapping to 7q11.23.

## PRODUCT

14-3-3  $\gamma$  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  $\mu$ M solution once resuspended using protocol below. Suitable for 50-100 transfections. Also see 14-3-3  $\gamma$  shRNA Plasmid (h): sc-29582-SH and 14-3-3  $\gamma$  shRNA (h) Lentiviral Particles: sc-29582-V as alternate gene silencing products.

For independent verification of 14-3-3  $\gamma$  (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-29582A, sc-29582B and sc-29582C.

## 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  $\mu$ l of the RNase-free water provided. Resuspension of the siRNA duplex in 330  $\mu$ l of RNase-free water makes a 10  $\mu$ M solution in a 10  $\mu$ M Tris-HCl, pH 8.0, 20 mM NaCl, 1 mM EDTA buffered solution.

## APPLICATIONS

14-3-3  $\gamma$  siRNA (h) is recommended for the inhibition of 14-3-3  $\gamma$  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  $\mu$ M in 66  $\mu$ 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  $\gamma$  (D-6): sc-398423 is recommended as a control antibody for monitoring of 14-3-3  $\gamma$  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 $\kappa$  BP-HRP: sc-516102 or m-IgG $\kappa$  BP-HRP (Cruz Marker): sc-516102-CM (dilution range: 1:1000-1:10000), Cruz Marker<sup>™</sup> Molecular Weight Standards: sc-2035, UltraCruz<sup>®</sup> Blocking Reagent: sc-516214 and Western Blotting Luminol Reagent: sc-2048. 2) Immunofluorescence: use m-IgG $\kappa$  BP-FITC: sc-516140 or m-IgG $\kappa$  BP-PE: sc-516141 (dilution range: 1:50-1:200) with UltraCruz<sup>®</sup> Mounting Medium: sc-24941 or UltraCruz<sup>®</sup> Hard-set Mounting Medium: sc-359850.

## RT-PCR REAGENTS

Semi-quantitative RT-PCR may be performed to monitor 14-3-3  $\gamma$  gene expression knockdown using RT-PCR Primer: 14-3-3  $\gamma$  (h)-PR: sc-29582-PR (20  $\mu$ l, 495 bp). Annealing temperature for the primers should be 55-60° C and the extension temperature should be 68-72° C.

## SELECT PRODUCT CITATIONS

- Aguilera, C., et al. 2006. Efficient nuclear export of p65-I $\kappa$ B $\alpha$  complexes requires 14-3-3 proteins. *J. Cell Sci.* 119: 3695-3704.
- Jin, Y., et al. 2006. 14-3-3 $\gamma$  binds to MDMX that is phosphorylated by UV-activated Chk1, resulting in p53 activation. *EMBO J.* 25: 1207-1218.
- Xu, Z., et al. 2013. 14-3-3 protein targets misfolded chaperone-associated proteins to aggregates. *J. Cell Sci.* 126: 4173-4186.
- Yoo, J.O., et al. 2016. miR-181b-3p promotes epithelial-mesenchymal transition in breast cancer cells through Snail stabilization by directly targeting YWHAG. *Biochim. Biophys. Acta* 1863: 1601-1611.

## RESEARCH USE

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