## SANTA CRUZ BIOTECHNOLOGY, INC.

# 14-3-3 ε siRNA (h): sc-29588



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  $\beta$ ,  $\gamma$ ,  $\varepsilon$ ,  $\zeta$ ,  $\eta$ ,  $\theta$  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

- 1. Morrison, D., et al. 1994. 14-3-3: modulators of signaling proteins? Science 266: 56-57.
- 2. 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.

## CHROMOSOMAL LOCATION

Genetic locus: YWHAE (human) mapping to 17p13.3.

## PRODUCT

14-3-3  $\epsilon$  siRNA (h) is a pool of 4 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  $\epsilon$  shRNA Plasmid (h): sc-29588-SH and 14-3-3  $\epsilon$  shRNA (h) Lentiviral Particles: sc-29588-V as alternate gene silencing products.

For independent verification of 14-3-3  $\epsilon$  (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-29588A, sc-29588B, sc-29588C and sc-29588D.

#### 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  $\varepsilon$  siRNA (h) is recommended for the inhibition of 14-3-3  $\varepsilon$  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  $\varepsilon$  (8C3): sc-23957 is recommended as a control antibody for monitoring of 14-3-3  $\varepsilon$  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).

#### **RT-PCR REAGENTS**

Semi-quantitative RT-PCR may be performed to monitor 14-3-3  $\epsilon$  gene expression knockdown using RT-PCR Primer: 14-3-3  $\epsilon$  (h)-PR: sc-29588-PR (20  $\mu$ l, 509 bp). Annealing temperature for the primers should be 55-60° C and the extension temperature should be 68-72° C.

#### SELECT PRODUCT CITATIONS

- 1. Aguilera, C., et al. 2006. Efficient nuclear export of p65-l $\kappa$ B $\alpha$  complexes requires 14-3-3 proteins. J. Cell Sci. 119: 3695-3704.
- 2. Tak, H., et al. 2007. 14-3-3 ε inhibits MK5-mediated cell migration by disrupting F-Actin polymerization. Cell. Signal. 19: 2379-2387.
- 3. Winter, S., et al. 2008. 14-3-3 proteins recognize a histone code at Histone H3 and are required for transcriptional activation. EMBO J. 27: 88-99.
- 4. Sorokina, E.M., et al. 2011. Intracellular targeting of peroxiredoxin 6 to lysosomal organelles requires MAPK activity and binding to 14-3-3  $\epsilon$ . Am. J. Physiol., Cell Physiol. 300: C1430-C1441.
- Nagappan, A., et al. 2013. *Helicobacter pylori* infection combined with DENA revealed altered expression of p53 and 14-3-3 isoforms in Gulo<sup>-/-</sup> mice. Chem. Biol. Interact. 206: 143-152.
- Cloutier, A., et al. 2018. hnRNP A1/A2 and Sam68 collaborate with SRSF10 to control the alternative splicing response to oxaliplatin-mediated DNA damage. Sci. Rep. 8: 2206.
- 7. Chapman, D.E., et al. 2019. Regulation of *in vivo* dynein force production by Cdk5 and 14-3-3  $\epsilon$  and KIAA0528. Nat. Commun. 10: 228.
- Holmes, T.R., et al. 2020. Targeting 14-3-3 ε-CDC25A interactions to trigger apoptotic cell death in skin cancer. Oncotarget 11: 3267-3278.

## **RESEARCH USE**

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