# SANTA CRUZ BIOTECHNOLOGY, INC.

# IRE1 $\alpha$ siRNA (h): sc-40705



### BACKGROUND

The accumulation of unfolded proteins within the endoplasmic recticulum (ER) of yeast and mammalian cells activates the unfolded protein response (UPR) pathway and leads to the transcription of ER-specific genes involved in protein folding. The activation of the UPR requires the ER transmembrane kinase IRE1p (for inositol-requiring and ER-to-nucleus signaling protein). IRE1 $\alpha$  and IRE1 $\beta$  are two mammalian homologs of the yeast IRE1p. These related proteins localize to the ER lumen and contain both a short transmembrane domain that spans the ER membrane and a cytosolic Ser/Thr kinase domain. IRE1 activation involves the oligomerization and transphosphorylation of the cytosolic portion of the proteins, which then potentiates its intrinsic kinase activity and, in turn, stimulates transcription of UPR-targeted genes. In response to stress, sensors for the ER mammalian cells activate IRE1 $\alpha$  and IRE1 $\beta$ , which then results in the phosphorylation of JNK (Jun N-Terminal Kinase) and the activation of the cellular MAP kinase pathway.

## REFERENCES

- Cox, J.S., et al. 1993. Transcriptional induction of genes encoding endoplasmic reticulum resident proteins requires a transmembrane protein kinase. Cell 73: 1197-1206.
- Welihinda, A.A., et al. 1997. Gene induction in response to unfolded protein in the endoplasmic reticulum is mediated through IRE1p kinase interaction with a transcriptional coactivator complex containing Ada5p. Proc. Natl. Acad. Sci. USA 94: 4289-4294.

#### CHROMOSOMAL LOCATION

Genetic locus: ERN1 (human) mapping to 17q23.3.

## PRODUCT

IRE1 $\alpha$  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 IRE1 $\alpha$  shRNA Plasmid (h): sc-40705-SH and IRE1 $\alpha$  shRNA (h) Lentiviral Particles: sc-40705-V as alternate gene silencing products.

For independent verification of IRE1 $\alpha$  (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-40705A, sc-40705B, sc-40705C and sc-40705D.

#### 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

 $\text{IRE1}\alpha$  siRNA (h) is recommended for the inhibition of  $\text{IRE1}\alpha$  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

IRE1 $\alpha$  (B-12): sc-390960 is recommended as a control antibody for monitoring of IRE1 $\alpha$  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 IRE1 $\alpha$  gene expression knockdown using RT-PCR Primer: IRE1 $\alpha$  (h)-PR: sc-40705-PR (20 µl, 461 bp). Annealing temperature for the primers should be 55-60° C and the extension temperature should be 68-72° C.

#### SELECT PRODUCT CITATIONS

- Zeng, L., et al. 2009. Sustained activation of XBP-1 splicing leads to endothelial apoptosis and atherosclerosis development in response to disturbed flow. Proc. Natl. Acad. Sci. USA 106: 8326-8331.
- Zhou, T., et al. 2015. RACK1 modulates apoptosis induced by sorafenib in HCC cells by interfering with the IRE1/XBP1 axis. Oncol. Rep. 33: 3006-3014.
- Zanotto-Filho, A., et al. 2016. Alkylating agent induced NRF2 blocks endoplasmic reticulum stress-mediated apoptosis via control of glutathione pools and protein thiol homeostasis. Mol. Cancer Ther. 15: 3000-3014.
- 4. Kim, TH., et al. 2017. The role of CREB3L4 in the proliferation of prostate cancer cells. Sci. Rep. 7: 45300.
- Shen, C., et al. 2018. The TLR4-IRE1α pathway activation contributes to palmitate-elicited lipotoxicity in hepatocytes. J. Cell. Mol. Med. 22: 3572-3581.
- Zhang, G., et al. 2019. Dual effects of gossypol on human hepatocellular carcinoma via endoplasmic reticulum stress and autophagy. Int. J. Biochem. Cell Biol. 113: 48-57.

## **RESEARCH USE**

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