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- Trockeneiszuschlag
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- Expressversand

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# IRE1 $\alpha$ siRNA (r): sc-270028

## BACKGROUND

The accumulation of unfolded proteins within the endoplasmic reticulum (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 trans-phosphorylation 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

1. Cox, J.S., et al. 1993. Transcriptional induction of genes encoding endoplasmic reticulum resident proteins requires a transmembrane protein kinase. *Cell* 73: 1197-1206.
2. 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 co-activator complex containing Ada5p. *Proc. Natl. Acad. Sci. USA* 94: 4289-4294.
3. Brewer, J.W., et al. 1997. A pathway distinct from the mammalian unfolded protein response regulates expression of endoplasmic reticulum chaperones in non-stressed cells. *EMBO J.* 16: 7207-7216.

## CHROMOSOMAL LOCATION

Genetic locus: Ern1 (rat) mapping to 10q32.1.

## PRODUCT

IRE1 $\alpha$  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  $\mu$ M solution once resuspended using protocol below. Suitable for 50-100 transfections. Also see IRE1 $\alpha$  shRNA Plasmid (r): sc-270028-SH and IRE1 $\alpha$  shRNA (r) Lentiviral Particles: sc-270028-V as alternate gene silencing products.

For independent verification of IRE1 $\alpha$  (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-270028A, sc-270028B and sc-270028C.

## 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

IRE1 $\alpha$  siRNA (r) is recommended for the inhibition of IRE1 $\alpha$  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  $\mu$ 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).

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 IRE1 $\alpha$  gene expression knockdown using RT-PCR Primer: IRE1 $\alpha$  (r)-PR: sc-270028-PR (20  $\mu$ l, 577 bp). Annealing temperature for the primers should be 55-60° C and the extension temperature should be 68-72° C.

## SELECT PRODUCT CITATIONS

1. Yao, W., et al. 2018. IRE1 $\alpha$  siRNA relieves endoplasmic reticulum stress-induced apoptosis and alleviates diabetic peripheral neuropathy *in vivo* and *in vitro*. *Sci. Rep.* 8: 2579.
2. Zheng, G., et al. 2019. Carbon monoxide releasing molecule-3 alleviates neuron death after spinal cord injury via inflammasome regulation. *EBioMedicine* 40: 643-654.
3. Hao, Y., et al. 2019. Unfolded protein response is involved in geniposide-regulating glucose-stimulated insulin secretion in INS-1 cells. *Cell Biochem. Funct.* 37: 368-376.

## RESEARCH USE

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