

IRE1 α 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 α and IRE1 β 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 α and IRE1 β , 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 α 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 IRE1 α shRNA Plasmid (r): sc-270028-SH and IRE1 α shRNA (r) Lentiviral Particles: sc-270028-V as alternate gene silencing products.

For independent verification of IRE1 α (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 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

IRE1 α siRNA (r) is recommended for the inhibition of IRE1 α 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

IRE1 α (B-12): sc-390960 is recommended as a control antibody for monitoring of IRE1 α 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 IRE1 α gene expression knockdown using RT-PCR Primer: IRE1 α (r)-PR: sc-270028-PR (20 μ 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 α 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.