

IRE1 β siRNA (h): sc-40707

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 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 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 coactivator 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.
4. Wang, X.Z., et al. 1998. Cloning of mammalian IRE1 reveals diversity in the ER stress responses. *EMBO J.* 17: 5708-5717.
5. Tirasophon, W., et al. 1998. A stress response pathway from the endoplasmic reticulum to the nucleus requires a novel bifunctional protein kinase/endoribonuclease (IRE1p) in mammalian cells. *Genes Dev.* 12: 1812-1824.
6. Harding, H.P., et al. 1999. Protein translation and folding are coupled by an endoplasmic-reticulum-resident kinase. *Nature* 397: 271-274.
7. Urano, F., et al. 2000. Coupling of stress in the ER to activation of JNK protein kinases by transmembrane protein kinase IRE1. *Science* 287: 664-666.

CHROMOSOMAL LOCATION

Genetic locus: ERN2 (human) mapping to 16p12.2.

RESEARCH USE

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

PROTOCOLS

See our web site at www.scbt.com for detailed protocols and support products.

PRODUCT

IRE1 β 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 μ M solution once resuspended using protocol below. Suitable for 50-100 transfections. Also see IRE1 β shRNA Plasmid (h): sc-40707-SH and IRE1 β shRNA (h) Lentiviral Particles: sc-40707-V as alternate gene silencing products.

For independent verification of IRE1 β (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-40707A, sc-40707B and sc-40707C.

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 β siRNA (h) is recommended for the inhibition of IRE1 β 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 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.

RT-PCR REAGENTS

Semi-quantitative RT-PCR may be performed to monitor IRE1 β gene expression knockdown using RT-PCR Primer: IRE1 β (h)-PR: sc-40707-PR (20 μ l, 578 bp). Annealing temperature for the primers should be 55-60° C and the extension temperature should be 68-72° C.