SANTA CRUZ BIOTECHNOLOGY, INC.

IRE1α siRNA (m): sc-40706

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The Power to Question

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). IRE1α and IRE1β 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 IRE1α and IRE1β, 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. 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 (mouse) mapping to 11 E1.

PRODUCT

IRE1α siRNA (m) 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 µM solution once resuspended using protocol below. Suitable for 50-100 transfections. Also see IRE1α shRNA Plasmid (m): sc-40706-SH and IRE1α shRNA (m) Lentiviral Particles: sc-40706-V as alternate gene silencing products.

For independent verification of IRE1α (m) gene silencing results, we also provide the individual siRNA duplex components. Each is available as 3.3 nmol of lyophilized siRNA. These include: sc-40706A, sc-40706B and sc-40706C.

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 µl of the RNAse-free water provided. Resuspension of the siRNA duplex in 330 µl of RNAse-free water makes a 10 µM solution in a 10 µM Tris-HCl, pH 8.0, 20 mM NaCl, 1 mM EDTA buffered solution.

APPLICATIONS

IRE1α siRNA (m) is recommended for the inhibition of IRE1α expression in mouse 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 µM in 66 µl. 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

IRE1α (B-12): sc-390960 is recommended as a control antibody for monitoring of IRE1α 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-IgGκ BP-HRP: sc-516102 or m-IgGκ 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-IgGκ BP-FITC: sc-516140 or m-IgGκ 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 IRE1α gene expression knockdown using RT-PCR Primer: IRE1α (m)-PR: sc-40706-PR (20 µl, 540 bp). Annealing temperature for the primers should be 55-60° C and the extension temperature should be 68-72° C.

SELECT PRODUCT CITATIONS

1. Kim, K.W., et al. 2010. Endoplasmic reticulum stress mediates radiation-induced autophagy by perk-eIF2α in caspase-3/7-deficient cells. Oncogene 29: 3241-3251.
2. Kim, S., et al. 2015. Endoplasmic reticulum stress-induced IRE1α activation mediates cross-talk of GSK-3β and XBP-1 to regulate inflammatory cytokine production. J. Immunol. 194: 4498-4506.
3. Li, W., et al. 2017. Phosphorylation of LAMP2A by p38 MAPK couples ER stress to chaperone-mediated autophagy. Nat. Commun. 8: 1763.
4. Kishino, A., et al. 2017. XBP1-FoxO1 interaction regulates ER stress-induced autophagy in auditory cells. Sci. Rep. 7: 4442.
5. Suzuki, R., et al. 2020. Intracellular accumulation of advanced glycation end-products induces osteoblast apoptosis via endoplasmic reticulum stress. J. Bone Miner. Res. 35: 1992-2003.

RESEARCH USE

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