



ERdj3 siRNA (m): sc-60596

BACKGROUND

Members of the heat shock protein 40 (HSP 40) family of proteins all contain a highly conserved J domain that associates with HSP 70 and regulates the function of HSP 70 by activating its adenosine triphosphatase activity. ERdj3, an HSP 40 chaperone, is expressed in the ER lumen, where it interacts with BiP, a molecule involved in retrotranslocating proteins out of the ER. ERdj3 also associates with several other protein substrates, including unfolded light chains, a nonsecreted Ig light chain mutant and a VSV-G ts045 mutant. Shiga toxin (Stx) is a bacterial tool that enzymatically inactivates the 28S rRNA, inhibiting protein synthesis of infected cells. Stx also interacts with ERdj3 and Sec 61 to form a complex through which proteins are retrotranslocated to the cytoplasm. ERdj3 may play a role in the ER quality control system.

REFERENCES

1. Yu, M., et al. 2000. HEDJ, an HSP 40 co-chaperone localized to the endoplasmic reticulum of human cells. *J. Biol. Chem.* 275: 24984-24992.
2. Meunier, L., et al. 2002. A subset of chaperones and folding enzymes form multiprotein complexes in endoplasmic reticulum to bind nascent proteins. *Mol. Biol. Cell* 13: 4456-4469.
3. Nakanishi, K., et al. 2004. Localization and function in endoplasmic reticulum stress tolerance of ERdj3, a new member of HSP 40 family protein. *Cell Stress Chaperones* 9: 253-264.
4. Shen, Y., et al. 2005. ERdj3, a stress-inducible endoplasmic reticulum DnaJ homologue, serves as a cofactor for BiP's interactions with unfolded substrates. *Mol. Biol. Cell* 16: 40-50.
5. Yu, M., et al. 2005. Shiga toxin is transported from the endoplasmic reticulum following interaction with the luminal chaperone HEDJ/ERdj3. *Infect. Immun.* 73: 2524-2532.

CHROMOSOMAL LOCATION

Genetic locus: Dnajb11 (mouse) mapping to 16 B1.

PRODUCT

ERdj3 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 μ M solution once resuspended using protocol below. Suitable for 50-100 transfections. Also see ERdj3 shRNA Plasmid (m): sc-60596-SH and ERdj3 shRNA (m) Lentiviral Particles: sc-60596-V as alternate gene silencing products.

For independent verification of ERdj3 (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-60596A, sc-60596B and sc-60596C.

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.

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

ERdj3 siRNA (m) is recommended for the inhibition of ERdj3 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 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

ERdj3 (C-7): sc-271240 is recommended as a control antibody for monitoring of ERdj3 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 ERdj3 gene expression knockdown using RT-PCR Primer: ERdj3 (m)-PR: sc-60596-PR (20 μ l). Annealing temperature for the primers should be 55-60° C and the extension temperature should be 68-72° C.