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EDEM siRNA (m): sc-143293

BACKGROUND

Proteins expressed in the endoplasmic reticulum (ER) are subjected to a tight quality control. Terminally misfolded proteins in the endoplasmic reticulum (ER) are retrotranslocated to the cytoplasm and degraded by proteasomes through a mechanism known as ER-associated degradation (ERAD). EDEM (ER degradation-enhancing α -mannosidase-like) protein is a type II membrane protein that localizes to the ER and is directly involved in ERAD. EDEM targets misfolded glycoproteins for degradation in an N-glycan-dependent manner and extracts misfolded glycoproteins from the calnexin cycle. The human EDEM gene maps to chromosome 3p.

REFERENCES

1. Hosokawa, N., et al. 2001. A novel ER α -mannosidase-like protein accelerates ER-associated degradation. *EMBO Rep.* 2: 415-422.
2. Oda, Y., et al. 2003. EDEM as an acceptor of terminally misfolded glycoproteins released from calnexin. *Science* 299: 1394-1397.
3. Yoshida, H., et al. 2003. A time-dependent phase shift in the mammalian unfolded protein response. *Dev. Cell* 4: 265-271.
4. Tardif, K.D., et al. 2004. Hepatitis C virus suppresses the IRE1-XBP1 pathway of the unfolded protein response. *J. Biol. Chem.* 279: 17158-17164.
5. Eriksson, K.K., et al. 2004. EDEM contributes to maintenance of protein folding efficiency and secretory capacity. *J. Biol. Chem.* 279: 44600-44605.
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7. Higgins, J.J., et al. 2004. Candidate genes for recessive non-syndromic mental retardation on chromosome 3p (MRT2A). *Clin. Genet.* 65: 496-500.
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CHROMOSOMAL LOCATION

Genetic locus: Edem1 (mouse) mapping to 6 E2.

PRODUCT

EDEM 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 EDEM shRNA Plasmid (m): sc-143293-SH and EDEM shRNA (m) Lentiviral Particles: sc-143293-V as alternate gene silencing products.

For independent verification of EDEM (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-143293A, sc-143293B and sc-143293C.

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

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

EDEM (D-1): sc-377394 is recommended as a control antibody for monitoring of EDEM 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 EDEM gene expression knockdown using RT-PCR Primer: EDEM (m)-PR: sc-143293-PR (20 μ l, 425 bp). Annealing temperature for the primers should be 55-60° C and the extension temperature should be 68-72° C.

RESEARCH USE

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