

EDEM2 siRNA (h): sc-77226

BACKGROUND

Proteins expressed in the endoplasmic reticulum (ER) are tightly regulated by a variety of quality control mechanisms. Terminally misfolded proteins in the ER are retrotranslocated to the cytoplasm and degraded by proteasomes through a mechanism known as ER-associated degradation (ERAD). EDEM2 (ER degradation-enhancing α -mannosidase-like 2) is a 578 amino acid secreted protein that, in conjunction with other EDEM proteins (namely EDEM and EDEM3), is involved in the ERAD pathway of protein degradation. EDEM2, a member of the glycosyl hydrolase 47 family, contains a mannosidase homology domain, an N-terminal cleavable signal sequence and a C-terminal extension that is required for both ER retention and the proper function of EDEM2. Human EDEM2 shares 93% sequence identity with its mouse homolog, suggesting a conserved role between species. Two isoforms of EDEM2 exist due to alternative splicing events.

REFERENCES

1. Online Mendelian Inheritance in Man, OMIM™. 2002. Johns Hopkins University, Baltimore, MD. MIM Number: 610302. World Wide Web URL: <http://www.ncbi.nlm.nih.gov/omim/>
2. Clark, H.F., et al. 2003. The secreted protein discovery initiative (SPDI), a large-scale effort to identify novel human secreted and transmembrane proteins: a bioinformatics assessment. *Genome Res.* 13: 2265-2270.
3. Mast, S.W., et al. 2005. Human EDEM2, a novel homolog of family 47 glycosidases, is involved in ER-associated degradation of glycoproteins. *Glycobiology* 15: 421-436.

CHROMOSOMAL LOCATION

Genetic locus: EDEM2 (human) mapping to 20q11.22.

PRODUCT

EDEM2 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 EDEM2 shRNA Plasmid (h): sc-77226-SH and EDEM2 shRNA (h) Lentiviral Particles: sc-77226-V as alternate gene silencing products.

For independent verification of EDEM2 (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-77226A, sc-77226B and sc-77226C.

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

EDEM2 siRNA (h) is recommended for the inhibition of EDEM2 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.

GENE EXPRESSION MONITORING

EDEM2 (JD-32): sc-130460 is recommended as a control antibody for monitoring of EDEM2 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 EDEM2 gene expression knockdown using RT-PCR Primer: EDEM2 (h)-PR: sc-77226-PR (20 μ l). Annealing temperature for the primers should be 55-60° C and the extension temperature should be 68-72° C.

SELECT PRODUCT CITATIONS

1. Munteanu, C.V.A., et al. 2021. Affinity proteomics and deglycoproteomics uncover novel EDEM2 endogenous substrates and an integrative ERAD network. *Mol. Cell. Proteomics* 20: 100125.

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.