SANTA CRUZ BIOTECHNOLOGY, INC.

EP58 siRNA (h): sc-105331

BACKGROUND

Soluble proteins in the endoplasmic reticulum (ER) contain a specific carboxy terminal sequence KDEL (Lys-Asp-Glu-Leu), and include the coat proteins required for vesicle budding from the ER, proteins that form retrograde vesicles on post-ER compartments, and integral membrane proteins that target vesicles to their correct destination. The retention of these soluble proteins in the ER depends on the interaction of the KDEL sequence with the corresponding KDEL receptor in the Golgi apparatus. When KDEL proteins reach the Golgi complex, they are recognized by the KDEL receptor and transported retrograde in COPI-coated vesicles back to the ER. A novel ligand ER protein 58 (EP58) shares no significant homology to any of the known ER-resident proteins. EP58 is primarily expressed in embryo, placenta, and adult heart. Sequence similarity to bacterial and fungus proteins suggests a possible role for EP58 in polysaccharide biosynthesis.

REFERENCES

1. Pelham, H.R. 1996. The dynamic organisation of the secretory pathway. Cell Struct. Funct. 21: 413-419.
2. Teasdale, R.D., et al. 1996. Signal-mediated sorting of membrane proteins between the endoplasmic reticulum and the golgi apparatus. Annu. Rev. Cell Dev. Biol. 12: 27-54.
3. Aoe, T., et al. 1997. The KDEL receptor, ERD2, regulates intracellular traffic by recruiting a GTPase-activating protein for ARF1. EMBO J. 16: 7305-7316.
4. Aoe, T., et al. 1998. Modulation of intracellular transport by transported proteins: insight from regulation of COPI-mediated transport. Proc. Natl. Acad. Sci. USA 95: 1624-1629.
5. Scheel, A.A., et al. 1998. Identification of amino acids in the binding pocket of the human KDEL receptor. J. Biol. Chem. 273: 2467-2472.
6. Kimata, Y., et al. 2000. Identification of a novel mammalian endoplasmic reticulum-resident KDEL protein using an EST database motif search. Gene 261: 321-327.
7. Murshid, A. and Presley, J.F. 2004. ER-to-Golgi transport and cytoskeletal interactions in animal cells. Cell. Mol. Life Sci. 61: 133-145.

CHROMOSOMAL LOCATION

Genetic locus: KDELC1 (human) mapping to 13q33.1.

PRODUCT

EP58 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 µM solution once resuspended using protocol below. Suitable for 50-100 transfections. Also see EP58 shRNA Plasmid (h): sc-105331-SH and EP58 shRNA (h) Lentiviral Particles: sc-105331-V as alternate gene silencing products.

For independent verification of EP58 (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-105331A, sc-105331B and sc-105331C.

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

EP58 siRNA (h) is recommended for the inhibition of EP58 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 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

EP58 (C-3): sc-390065 is recommended as a control antibody for monitoring of EP58 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 EP58 gene expression knockdown using RT-PCR Primer: EP58 (h)-PR: sc-105331-PR (20 µl). 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.

PROTOCOLS

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