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ERp57 siRNA (h2): sc-44299

BACKGROUND

Mammals defend themselves against intracellular pathogens through presentation of cytoplasmically-derived short pathogenic peptides to the cell surface of cytotoxic T lymphocytes, which subsequently leads to cytotoxic events with respect to the affected cell. Antigen presentation is mediated by major histocompatibility complex (MHC) class I molecules, which bind and coordinate short pathogenic peptides. MHC class I molecules assemble in the endoplasmic reticulum with chaperones before binding to the transporter associated with antigen processing (TAP). ERp57, also designated GRP57, GRP58, ERp60 and ERp61, is a component of the MHC class I pathway that appears to interact with MHC class I molecules before they associate with TAP. The human ERp57 gene maps to chromosome 15q15.3 and encodes a 505 amino acid protein. ERp57 has two Trp-Cys-Gly-His-Cys-Lys motifs completely conserved among the mammals. ERp57 may act as a protease, a protein disulfide isomerase, a phospholipase or a combination of these.

REFERENCES

- Hirano, N., et al. 1995. Molecular cloning of the human glucose-regulated protein ERp57/GRP58, a thiol-dependent reductase. Identification of its secretory form and inducible expression by the oncogenic transformation. *Eur. J. Biochem.* 234: 336-342.
- Hughes, E.A. and Cresswell, P. 1998. The thiol oxidoreductase ERp57 is a component of the MHC class I peptide-loading complex. *Curr. Biol.* 8: 709-712.
- Morrice, N.A. and Powis, S.J. 1998. A role for the thiol-dependent reductase ERp57 in the assembly of MHC class I molecules. *Curr. Biol.* 8: 713-716.
- Online Mendelian Inheritance in Man, OMIM[™]. 1998. Johns Hopkins University, Baltimore, MD. MIM Number: 602046. World Wide Web URL: <http://www.ncbi.nlm.nih.gov/omim/>
- MacAry, P.A., et al. 2001. Mobilization of MHC class I molecules from late endosomes to the cell surface following activation of CD34-derived human Langerhans cells. *Proc. Natl. Acad. Sci. USA* 98: 3982-3987.

CHROMOSOMAL LOCATION

Genetic locus: PDIA3 (human) mapping to 15q15.3.

PRODUCT

ERp57 siRNA (h2) 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 ERp57 shRNA Plasmid (h2): sc-44299-SH and ERp57 shRNA (h2) Lentiviral Particles: sc-44299-V as alternate gene silencing products.

For independent verification of ERp57 (h2) gene silencing results, we also provide the individual siRNA duplex components. Each is available as 3.3 nmol of lyophilized siRNA. These include: sc-44299A, sc-44299B and sc-44299C.

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

ERp57 siRNA (h2) is recommended for the inhibition of ERp57 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

ERp57 (MaP.ERp57): sc-23886 is recommended as a control antibody for monitoring of ERp57 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 ERp57 gene expression knockdown using RT-PCR Primer: ERp57 (h)-PR: sc-35341-PR (20 μ l, 451 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.