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ERp57 shRNA (h2) Lentiviral Particles: sc-44299-V

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 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/GRP 58, 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.

STORAGE

Store lentiviral particles at -80° C. Stable for at least one year from the date of shipment. Once thawed, particles can be stored at 4° C for up to one week. Avoid repeated freeze thaw cycles.

RESEARCH USE

The purchase of this product conveys to the buyer the nontransferable right to use the purchased amount of the product and all replicates and derivatives for research purposes conducted by the buyer in his laboratory only (whether the buyer is an academic or for-profit entity). The buyer cannot sell or otherwise transfer (a) this product (b) its components or (c) materials made using this product or its components to a third party, or otherwise use this product or its components or materials made using this product or its components for Commercial Purposes.

PRODUCT

ERp57 shRNA (h2) Lentiviral Particles is a pool of concentrated, transduction-ready viral particles containing 3 target-specific constructs that encode 19-25 nt (plus hairpin) shRNA designed to knock down gene expression. Each vial contains 200 µl frozen stock containing 1.0×10^6 infectious units of virus (IFU) in Dulbecco's Modified Eagle's Medium with 25 mM HEPES pH 7.3. Suitable for 10-20 transductions. Also see ERp57 siRNA (h2): sc-44299 and ERp57 shRNA Plasmid (h2): sc-44299-SH as alternate gene silencing products.

APPLICATIONS

ERp57 shRNA (h2) Lentiviral Particles is recommended for the inhibition of ERp57 expression in human cells.

SUPPORT REAGENTS

Control shRNA Lentiviral Particles: sc-108080. Available as 200 µl frozen viral stock containing 1.0×10^6 infectious units of virus (IFU); contains an shRNA construct encoding a scrambled sequence that will not lead to the specific degradation of any known cellular mRNA.

GENE EXPRESSION MONITORING

ERp57 (B-5): sc-166680 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 (secondary) reagents are recommended: 1) Western Blotting: use goat anti-mouse IgG-HRP: sc-2005 (dilution range: 1:2000-1:32,000) or Cruz Marker™ compatible goat anti-mouse IgG-HRP: sc-2031 (dilution range: 1:2000-1:5000), Cruz Marker™ Molecular Weight Standards: sc-2035, TBS Blotto A Blocking Reagent: sc-2333 and Western Blotting Luminol Reagent: sc-2048. 2) Immunofluorescence: use goat anti-mouse IgG-FITC: sc-2010 (dilution range: 1:100-1:400) or goat anti-mouse IgG-TR: sc-2781 (dilution range: 1:100-1:400) with UltraCruz™ Mounting Medium: sc-24941.

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 µl, 451 bp). Annealing temperature for the primers should be 55-60° C and the extension temperature should be 68-72° C.

BIOSAFETY

Lentiviral particles can be employed in standard Biosafety Level 2 tissue culture facilities (and should be treated with the same level of caution as with any other potentially infectious reagent). Lentiviral particles are replication-incompetent and are designed to self-inactivate after transduction and integration of shRNA constructs into genomic DNA of target cells.

PROTOCOLS

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