SANTA CRUZ BIOTECHNOLOGY, INC.

GILT siRNA (h): sc-39522

BACKGROUND

Proteins internalized into the endocytic pathway are usually degraded. Efficient proteolysis requires denaturation, induced by acidic conditions within lysosomes, and reduction of inter- and intrachain disulfide bonds. Cytosolic reduction is mediated enzymatically by thioredoxin. In the endocytic pathway, reduction of protein disulfide bonds is important for the generation of MHC class II-peptide complexes. This process is catalyzed by a γ-interferon-inducible thiol reductase (GILT). GILT is synthesized as a precursor, and following delivery to MHC class II-containing compartments (MIICs), is processed to the mature form via cleavage of amino- and carboxy-terminal propeptides. A lysosomal thiol reductase, GILT, is optimally active at low pH and capable of catalyzing disulfide bond reduction both *in vivo* and *in vitro*. GILT is expressed constitutively in antigen-presenting cells and is induced by γ-interferon in other cell types, suggesting a potentially important role in antigen processing. Additionally, T cell recognition of select exogenous and endogenous epitopes is dependent on tumor cell expression of GILT. The absence of GILT in melanomas alters antigen processing and the hierarchy of immunodominant epitope presentation.

REFERENCES

1. Cresswell, P., Arunachalam, B., Bangia, N., Dick, T., Diedrich, G., Hughes, E. and Maric, M. 1999. Thiol oxidation and reduction in MHC-restricted antigen processing and presentation. Immunol. Res. 19: 191-200.
2. Phan, U.T., Arunachalam, B. and Cresswell, P. 2000. γ-interferon-inducible lysosomal thiol reductase (GILT). Maturation, activity, and mechanism of action. J. Biol. Chem. 275: 25907-25914.
3. Arunachalam, B., Phan, U.T., Geuze, H.J. and Cresswell, P. 2000. Enzymatic reduction of disulfide bonds in lysosomes: characterization of a γ-interferon-inducible lysosomal thiol reductase (GILT). Proc. Natl. Acad. Sci. USA 97: 745-750.
4. Haque, M.A., Li, P., Jackson, S.K., Zarour, H.M., Hawes, J.W., Phan, U.T., Maric, M., Cresswell, P. and Blum, J.S. 2002. Absence of γ-interferon-inducible lysosomal thiol reductase in melanomas disrupts T cell recognition of select immunodominant epitopes. J. Exp. Med. 195: 1267-1277.
5. LocusLink Report (LocusID: 604664). http://www.ncbi.nlm.nih.gov/LocusLink/

CHROMOSOMAL LOCATION

Genetic locus: IFI30 (human) mapping to 19p13.11.

PRODUCT

GILT 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 GILT shRNA Plasmid (h): sc-39522-SH and GILT shRNA (h) Lentiviral Particles: sc-39522-V as alternate gene silencing products.

For independent verification of GILT (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-39522A, sc-39522B and sc-39522C.

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

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

GILT (G-11): sc-393507 is recommended as a control antibody for monitoring of GILT 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 GILT gene expression knockdown using RT-PCR Primer: GILT (h)-PR: sc-39522-PR (20 µl, 521 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.

PROTOCOLS

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