

TPN siRNA (h): sc-42986

BACKGROUND

TPN (tapasin, TPSN, TAPBP, transporter associated with antigen processing-A, TAP-A) is a type I membrane glycoprotein whose cDNA maps to chromosome 6p21.32 and encodes a 488 residue protein. Phosphorylation of TAP (transporter associated with antigen processing), a heterodimer consisting of TAP1 and TAP2, causes the assembly of high molecular weight complexes which contain TPN and facilitate the transfer of peptide antigens onto major histocompatibility complex class I (MHC1) molecules. TPN mediates the association of newly assembled MHC1 molecules with TAP and controls antigen loading in the lumen of the endoplasmic reticulum. The cytoplasmic portion of TPN contains a double-lysine motif (-KKKAE-COOH) that is believed to mediate retention in the endoplasmic reticulum. TPN knockout mice show defects in the cell surface expression of MHC1 molecules, antigen presentation to CD8⁺ T cells, and other humoral responses, suggesting that TPN is important for retention of empty MHC1 molecules in the ER.

REFERENCES

1. Li, S., et al. 1997. Cloning and functional characterization of a subunit of the transporter associated with antigen processing. *Proc. Natl. Acad. Sci. USA* 94: 8708-8713.
2. Ortmann, B., et al. 1997. A critical role for tapasin in the assembly and function of multimeric MHC class I-TAP complexes. *Science* 277: 1306-1309.
3. Li, S., et al. 1999. Peptide-bound major histocompatibility complex class I molecules associate with tapasin before dissociation from transporter associated with antigen processing. *J. Biol. Chem.* 274: 8649-8654.
4. Grandea, A.G., et al. 2000. Impaired assembly yet normal trafficking of MHC class I molecules in tapasin mutant mice. *Immunity* 13: 213-222.
5. Li, S., et al. 2000. Tapasin is required for efficient peptide binding to transporter associated with antigen processing. *J. Biol. Chem.* 275: 1581-1586.
6. Li, Y., et al. 2000. Regulation of transporter associated with antigen processing by phosphorylation. *J. Biol. Chem.* 275: 24130-24135.

CHROMOSOMAL LOCATION

Genetic locus: TAPBP (human) mapping to 6p21.32.

PRODUCT

TPN 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 TPN shRNA Plasmid (h): sc-42986-SH and TPN shRNA (h) Lentiviral Particles: sc-42986-V as alternate gene silencing products.

For independent verification of TPN (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-42986A, sc-42986B and sc-42986C.

RESEARCH USE

For research use only, not for use in diagnostic procedures.

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

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

TPN (E-11): sc-393552 is recommended as a control antibody for monitoring of TPN 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 TPN gene expression knockdown using RT-PCR Primer: TPN (h)-PR: sc-42986-PR (20 μ l). Annealing temperature for the primers should be 55-60° C and the extension temperature should be 68-72° C.

PROTOCOLS

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