SANTA CRUZ BIOTECHNOLOGY, INC.

TIP47 siRNA (h): sc-44157

BACKGROUND

Tail-interacting 47kDa protein (TIP47), known also as human placental tissue protein 17b (PP17b), binds to cytoplasmic domains of the cation-dependent (CD) and cation-independent (CI) mannose 6-phosphate receptors (MPRs) and facilitates their transport from endosomes to the Golgi complex. The inability of TIP47 to bind several proteins also transported from endosomes to the *trans* Golgi network indicates that TIP47 associates with a very select set of cargo molecules. In CD-MPR, TIP47 recognizes a phenylalanine/tryptophan signal sequence essential for proper sorting within the endosomal pathway. For CI-MPR binding, TIP47 requires cytoplasmic residues 48-74 of CI-MPR for high-affinity binding while residues 75-163 of CI-MPR aid in the presentation of the higher-affinity residues. Additionally, TIP47 competes with AP-2 clathrin adaptor for binding residues 24-29 of CI-MPR. In tissue extracts of cervical carcinoma patients, TIP47 is overexpressed. Dysplastic cells in high grade dysplasias express more TIP47 than dysplastic cells in low grade dysplasias, and both cytoplasmic types of dysplasias express more TIP47 than normal cervical epithelial cells. The gene encoding human TIP47 maps to chromosome 19p13.3.

REFERENCES

1. Diaz, E. and Pfeffer, S.R. 1998. TIP47: a cargo selection device for mannose 6-phosphate receptor trafficking. Cell 93: 433-443.
2. Than, N.G., et al. 1998. Cloning and sequence analysis of cDNAs encoding human placental tissue protein 17 (PP17) variants. Eur. J. Biochem. 258: 752-757.
3. Orsel, J.G., et al. 2000. Recognition of the 300 kDa mannose 6-phosphate receptor cytoplasmic domain by 47 kDa tail-interacting protein. Proc. Natl. Acad. Sci. USA 97: 9047-9051.
4. Krise, J.P., et al. 2000. Quantitative analysis of TIP47-receptor cytoplasmic domain interactions: implications for endosome-to-*trans* Golgi network trafficking. J. Biol. Chem. 275: 25188-25193.
5. Than, G.N., et al. 2001. Overexpression of placental tissue protein 17b/TIP47 in cervical dysplasias and cervical carcinoma. Anticancer Res. 21: 639-642.

CHROMOSOMAL LOCATION

Genetic locus: PLIN3 (human) mapping to 19p13.3.

PRODUCT

TIP47 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 TIP47 shRNA Plasmid (h): sc-44157-SH and TIP47 shRNA (h) Lentiviral Particles: sc-44157-V as alternate gene silencing products.

For independent verification of TIP47 (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-44157A, sc-44157B and sc-44157C.

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

TIP47 siRNA (h) is recommended for the inhibition of TIP47 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

TIP47 (B-3): sc-390981 is recommended as a control antibody for monitoring of TIP47 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 TIP47 gene expression knockdown using RT-PCR Primer: TIP47 (h)-PR: sc-44157-PR (20 µl, 597 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.