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TIP47 shRNA (h) Lentiviral Particles: sc-44157-V

BACKGROUND

Tail-interacting 47 kDa 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.

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.

CHROMOSOMAL LOCATION

Genetic locus: M6PRBP1 (human) mapping to 19p13.3.

PRODUCT

TIP47 shRNA (h) 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 μ l 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 TIP47 siRNA (h): sc-44157 and TIP47 shRNA Plasmid (h): sc-44157-SH as alternate gene silencing products.

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.

APPLICATIONS

TIP47 shRNA (h) Lentiviral Particles is recommended for the inhibition of TIP47 expression in human cells.

SUPPORT REAGENTS

Control shRNA Lentiviral Particles: sc-108080. Available as 200 μ l 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

TIP47 (E-5): sc-393461 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 (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 TIP47 gene expression knockdown using RT-PCR Primer: TIP47 (h)-PR: sc-44157-PR (20 μ l, 597 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.

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.

PROTOCOLS

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