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TPR shRNA (h) Lentiviral Particles: sc-45343-V

BACKGROUND

The vertebrate nuclear pore complex (NPC) is a macromolecular assembly of protein subcomplexes forming a structure of eightfold radial symmetry. The NPC core consists of globular subunits sandwiched between two coaxial ring-like structures of which the ring facing the nuclear interior is capped by a fibrous structure called the nuclear basket. The assembly of the NPC is a stepwise process in which Trp-containing peripheral structures assemble after other components, including p62. TPR localizes to intranuclear filaments of the NPC, and is a component of the cytoplasmic fibrils of the NPC implicated in nuclear protein import. Experimental data suggest that TPR is tethered to intranuclear filaments of the NPC by its coiled coil domain leaving the acidic COOH terminus free to interact with soluble transport factors and mediate export of macromolecules from the nucleus.

REFERENCES

1. Byrd, D.A., et al. 1994. Tpr, a large coiled coil protein whose amino terminus is involved in activation of oncogenic kinases, is localized to the cytoplasmic surface of the nuclear pore complex. *J. Cell Biol.* 127: 1515-1526.
2. Bangs, P., et al. 1998. Functional analysis of Tpr: identification of nuclear pore complex association and nuclear localization domains and a role in mRNA export. *J. Cell Biol.* 143: 1801-1812.
3. Cordes, V.C., et al. 1998. Molecular segments of protein Tpr that confer nuclear targeting and association with the nuclear pore complex. *Exp. Cell Res.* 245: 43-56.
4. Krull, S. et al. 2004. Nucleoporins as components of the nuclear pore complex core structure and Tpr as the architectural element of the nuclear basket. *Mol. Biol. Cell.* 15: 4261-4277.
5. Beausoleil, S.A. et al. 2004. Large-scale characterization of HeLa cell nuclear phosphoproteins. *Proc. Natl. Acad. Sci. USA* 101: 12130-12135.

CHROMOSOMAL LOCATION

Genetic locus: TPR (human) mapping to 1q31.1.

PRODUCT

TPR 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 TPR siRNA (h): sc-45343 and TPR shRNA Plasmid (h): sc-45343-SH as alternate gene silencing products.

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.

APPLICATIONS

TPR shRNA (h) Lentiviral Particles is recommended for the inhibition of TPR 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

TPR (H-12): sc-271317 is recommended as a control antibody for monitoring of TPR 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 TPR gene expression knockdown using RT-PCR Primer: TPR (h)-PR: sc-45343-PR (20 μ l). Annealing temperature for the primers should be $55-60^{\circ}$ C and the extension temperature should be $68-72^{\circ}$ 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.

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.