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TPR siRNA (h): sc-45343

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 TPR-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.

CHROMOSOMAL LOCATION

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

PRODUCT

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

For independent verification of TPR (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-45343A, sc-45343B and sc-45343C.

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

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

TPR (H-8): sc-271565 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 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 TPR gene expression knockdown using RT-PCR Primer: TPR (h)-PR: sc-45343-PR (20 μ l, 318 bp). Annealing temperature for the primers should be 55-60° C and the extension temperature should be 68-72° C.

SELECT PRODUCT CITATIONS

- Nakano, H., et al. 2010. Nucleoporin translocated promoter region (TPR) associates with dynein complex, preventing chromosome lagging formation during mitosis. *J. Biol. Chem.* 285: 10841-10849.
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- Kobayashi, A., et al. 2015. Therapeutic potential of mitotic interaction between the nucleoporin TPR and aurora kinase A. *Cell Cycle* 14: 1447-1458.
- Zhang, P., et al. 2016. Identification of replication-dependent and replication-independent linker histone complexes: TPR specifically promotes replication-dependent linker histone stability. *BMC Biochem.* 17: 18.
- Dewi, F.R.P., et al. 2018. Colorectal cancer cells require glycogen synthase kinase-3 β for sustaining mitosis via translocated promoter region (TPR)-dynein interaction. *Oncotarget* 9: 13337-13352.
- Hazawa, M., et al. 2018. ROCK-dependent phosphorylation of NUP62 regulates p63 nuclear transport and squamous cell carcinoma proliferation. *EMBO Rep.* 19: 73-88.
- Mistriotis, P., et al. 2019. Confinement hinders motility by inducing RhoA-mediated nuclear influx, volume expansion, and blebbing. *J. Cell Biol.* E-published.

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.