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TPR siRNA (m) : sc-45344

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.

REFERENCES

- 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.
- 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.
- 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.
- 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.
- 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 (mouse) mapping to 1 G1.

PRODUCT

TPR siRNA (m) 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 (m): sc-45344-SH and TPR shRNA (m) Lentiviral Particles: sc-45344-V as alternate gene silencing products.

For independent verification of TPR (m) gene silencing results, we also provide the individual siRNA duplex components. Each is available as 3.3 nmol of lyophilized siRNA. These include: sc-45344A, sc-45344B and sc-45344C.

PROTOCOLS

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

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 (m) is recommended for the inhibition of TPR expression in mouse 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 (m)-PR: sc-45344-PR (20 μ l). 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.