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# TCP-1 $\theta$ shRNA (m) Lentiviral Particles: sc-43452-V

## BACKGROUND

The protein TCP-1 (t complex polypeptide 1) is a subunit of the hetero-oligomeric complex CCT (chaperonin containing TCP-1) present in the eukaryotic cytosol. The CCT of eukaryotic cytosol is composed of eight different subunit species, TCP-1  $\alpha$ ,  $\beta$ ,  $\gamma$ ,  $\delta$ ,  $\epsilon$ ,  $\zeta$ ,  $\eta$  and  $\theta$ , each encoded by a different gene. Two  $\zeta$  subunits have been described: TCP-1  $\zeta$  (also designated TCP-1  $\zeta$ 1) and TCP-1  $\zeta$ 2. TCP-1 subunits are proposed to have independent functions in folding its *in vivo* substrates, the actins and tubulins. TCP-1 was first identified in the mouse as relevant for tail-less and embryonic lethal phenotypes. Sequences homologous to TCP-1 have been isolated in several other species, and the yeast TCP-1 has been shown to encode a molecular chaperone for Actin and Tubulin. TCP-1 found in mammalian cells and yeast plays an important role in the folding of cytosolic proteins.

## REFERENCES

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- Iijima, M., et al. 1998. A *Dictyostelium discoideum* homologue to TCP-1 is essential for growth and development. *Gene* 213: 101-106.
- Ritco-Vonsovici, M., et al. 2000. Defining the eukaryotic cytosolic chaperonin-binding sites in human tubulins. *J. Mol. Biol.* 304: 81-98.
- Hynes, G.M., et al. 2000. Individual subunits of the eukaryotic cytosolic chaperonin mediate interactions with binding sites located on subdomains of  $\beta$ -Actin. *J. Biol. Chem.* 275: 18985-18994.
- Campos, E.G., et al. 2000. Cloning of the chaperonin t complex polypeptide 1 gene from *Schistosoma mansoni* and studies of its expression levels under heat shock and oxidative stress. *Parasitol. Res.* 86: 253-258.
- Yokota, S.I., et al. 2000. Upregulation of cytosolic chaperonin CCT subunits during recovery from chemical stress that causes accumulation of unfolded proteins. *Eur. J. Biochem.* 267: 1658-1664.

## CHROMOSOMAL LOCATION

Genetic locus: Cct8 (mouse) mapping to 16 C3.3.

## PRODUCT

TCP-1  $\theta$  shRNA (m) 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  $\mu$ l frozen stock containing  $1.0 \times 10^6$  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 TCP-1  $\theta$  siRNA (m): sc-43452 and TCP-1  $\theta$  shRNA Plasmid (m): sc-43452-SH as alternate gene silencing products.

## STORAGE

Store lentiviral particles at  $-80^\circ$  C. Stable for at least one year from the date of shipment. Once thawed, particles can be stored at  $4^\circ$  C for up to one week. Avoid repeated freeze thaw cycles.

## APPLICATIONS

TCP-1  $\theta$  shRNA (m) Lentiviral Particles is recommended for the inhibition of TCP-1  $\theta$  expression in mouse cells.

## SUPPORT REAGENTS

Control shRNA Lentiviral Particles: sc-108080. Available as 200  $\mu$ l frozen viral stock containing  $1.0 \times 10^6$  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

TCP-1  $\theta$  (E-1): sc-377453 is recommended as a control antibody for monitoring of TCP-1  $\theta$  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 TCP-1  $\theta$  gene expression knockdown using RT-PCR Primer: TCP-1  $\theta$  (m)-PR: sc-43452-PR (20  $\mu$ 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.

## PROTOCOLS

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