SANTA CRUZ BIOTECHNOLOGY, INC.

TDP1 siRNA (h): sc-41056

BACKGROUND

Tyrosyl-DNA phosphodiesterase 1 (TDP1), a DNA repair enzyme, catalyzes the hydrolysis of phophodiester bonds between tyrosine residues and DNA 3'-phosphates. In addition, TDP1 removes glycolate from single-stranded DNA containing a 3'-phosphoglycolate, suggesting a role in repair of free-radical mediated DNA double-strand breaks. A unique HKD signature motif with highly conserved lysine and histidine residues present in TDP1 places the enzyme in a distinct class within the phospholipase D superfamily. The hydrolytic reaction catalyzed by TDP1 occurs by a phosphoryl transfer reaction common to all members of the PLD superfamily. Loss-of-function mutations in TDP1 may cause spinocerebellar ataxia with axonal neuropathy by interfering with DNA transcription or by inducing apoptosis in postmitotic neurons.

REFERENCES

1. Interthal, H., et al. 2001. The tyrosyl-DNA phosphodiesterase TDP1 is a member of the phopholipase D superfamily. Proc. Natl. Acad. Sci. USA 98: 12009-12014.
2. Davies, D.R., et al. 2002. Insights into substrate binding and catalytic mechanism of human tyrosyl-DNA phophodiesterase (TDP1) from vanadate and tungstate-inhibited structures. J. Mol. Biol. 324: 917-932.
3. Inamdar, K.V., et al. 2002. Conversion of phosphoglycolate to phosphate termini on 3' overhangs of DNA double strand breaks by the human tyrosyl-DNA phosphodiesterase hTDP1. J. Biol. Chem. 277: 27162-27168.
4. Takashima, H., et al. 2002. Mutation of TDP1, encoding a topoisomerase I-dependent DNA damage repair enzyme in spinocerebellar ataxia with axonal neuropathy. Nat. Genet. 32: 267-272.

CHROMOSOMAL LOCATION

Genetic locus: TDP1 (human) mapping to 14q32.11.

PRODUCT

TDP1 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 µM solution once resuspended using protocol below. Suitable for 50-100 transfections. Also see TDP1 shRNA Plasmid (h): sc-41056-SH and TDP1 shRNA (h) Lentiviral Particles: sc-41056-V as alternate gene silencing products.

For independent verification of TDP1 (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-41056A, sc-41056B and sc-41056C.

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 µl of the RNAse-free water provided. Resuspension of the siRNA duplex in 330 µl of RNAse-free water makes a 10 µM solution in a 10 µM Tris-HCl, pH 8.0, 20 mM NaCl, 1 mM EDTA buffered solution.

APPLICATIONS

TDP1 siRNA (h) is recommended for the inhibition of TDP1 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 µM in 66 µl. 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

TDP1 (C-3): sc-365674 is recommended as a control antibody for monitoring of TDP1 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-IgGκ BP-HRP: sc-516102 or m-IgGκ 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-IgGκ BP-FITC: sc-516140 or m-IgGκ 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 TDP1 gene expression knockdown using RT-PCR Primer: TDP1 (h)-PR: sc-41056-PR (20 µl). Annealing temperature for the primers should be 55-60° C and the extension temperature should be 68-72° C.

SELECT PRODUCT CITATIONS

1. Zhou, T., et al. 2009. Tyrosyl-DNA phosphodiesterase and the repair of 3'-phosphoglycolate-terminated DNA double-strand breaks. DNA Repair 8: 901-911.
2. Jensen, P.W., et al. 2013. Real-time detection of TDP1 activity using a fluorophore-quencher coupled DNA-biosensor. Biosens. Bioelectron. 48: 230-237.

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.