

▶ Trx-2 siRNA (m): sc-60084

BACKGROUND

Thioredoxin (Trx) is a redox protein found in several species, such as bacteria, plants and mammals, and contains a conserved active site consisting of Trp-Cys-Gly-Pro-Cys. Trx has several biological functions. It acts as a hydrogen donor for ribonucleotide reductase, which is critical for DNA synthesis, and modulates the DNA-binding activity of several transcription factors, including NFκB, AP-1, p53, TFIIIC and glucocorticoid receptor. Trx also stimulates cell growth, is an inhibitor of apoptosis and plays a role in the protection against oxidative stress. Drugs that inhibit Trx have antitumor activity, suggesting that Trx is involved in a variety of human diseases, including cancer. Thioredoxin 2 (Trx-2) is a small redox protein that is localized to the mitochondria and is essential for cell viability, playing a crucial role in the scavenging of Ros in mitochondria and regulating the mitochondrial apoptosis signaling pathway. Trx reductases (TrxR1 and TrxR2) are ubiquitously expressed flavoproteins that catalyze the NADPH-dependent reduction of Trx as well as several other oxidized cellular components. Mammalian Trx reductases are a part of a selenium-containing pyridine nucleotide-disulphide oxidoreductase family, which has a conserved catalytic site of Cys-Val-Asn-Val-Gly-Cys. TrxR1 and TrxR2 are also involved in the prevention of oxidative stress. Inhibition of TrxR activity may provide for potential treatments of cancer, AIDS and other autoimmune diseases as well as bacterial infections and parasitic diseases.

REFERENCES

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2. Tanaka, T., et al. 2000. Redox regulation by thioredoxin superfamily; protection against oxidative stress and aging. *Free Radic. Res.* 33: 851-855.
3. Arner, E.S., et al. 2000. Physiological functions of thioredoxin and thioredoxin reductase. *Eur. J. Biochem.* 267: 6102-6109.
4. Williams, C.H., et al. 2000. Thioredoxin reductase two modes of catalysis have evolved. *Eur. J. Biochem.* 267: 6110-6117.
5. Becker, K., et al. 2000. Thioredoxin reductase as a pathophysiological factor and drug target. *Eur. J. Biochem.* 267: 6118-6125.
6. Mustacich, D., et al. 2000. Thioredoxin reductase. *Biochem. J.* 346: 1-8.

CHROMOSOMAL LOCATION

Genetic locus: Txn2 (mouse) mapping to 15 E1.

PRODUCT

Trx-2 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 μM solution once resuspended using protocol below. Suitable for 50-100 transfections. Also see Trx-2 shRNA Plasmid (m): sc-60084-SH and Trx-2 shRNA (m) Lentiviral Particles: sc-60084-V as alternate gene silencing products.

For independent verification of Trx-2 (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-60084A, sc-60084B and sc-60084C.

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

Trx-2 shRNA Plasmid (m) is recommended for the inhibition of Trx-2 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 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

Trx-2 (F-10): sc-133201 is recommended as a control antibody for monitoring of Trx-2 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 Trx-2 gene expression knockdown using RT-PCR Primer: Trx-2 (m)-PR: sc-60084-PR (20 μl, 588 bp). 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.

PROTOCOLS

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