

Nox4 siRNA (r): sc-61887

BACKGROUND

The superoxide-generating NADPH oxidase includes a membrane-bound flavocytochrome containing two subunits, gp91-phox and p22-phox, and the cytosolic proteins p47-phox and p67-phox. During activation of the NADPH oxidase, p47-phox and p67-phox migrate to the plasma membrane where they associate with the flavocytochrome, cytochrome b558, to form the active enzyme complex. The p22 and gp91-phox subunits also function as surface O₂ sensors that initiate cellular signaling in response to hypoxic conditions. Nox4 (also known as Renox) is a renal gp91-phox homolog highly expressed at the site of erythropoietin production in the proximal convoluted tubule epithelial cells of the renal cortex. Nox4 is also expressed in fetal tissues, placenta, glioblastoma and vascular cells. Like gp91-phox, the enzymatic activity of Nox4 produces superoxide anions. In vascular cells, the addition of Angiotensin II increases Nox4 expression, which suggests a role for Nox4 in vascular oxidative stress response. The gene encoding human Nox4 maps to chromosome 11q14.3.

CHROMOSOMAL LOCATION

Genetic locus: Nox4 (rat) mapping to 1q32.

PRODUCT

Nox4 siRNA (r) 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 Nox4 shRNA Plasmid (r): sc-61887-SH and Nox4 shRNA (r) Lentiviral Particles: sc-61887-V as alternate gene silencing products.

For independent verification of Nox4 (r) gene silencing results, we also provide the individual siRNA duplex components. Each is available as 3.3 nmol of lyophilized siRNA. These include: sc-61887A, sc-61887B and sc-61887C.

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

Nox4 siRNA (r) is recommended for the inhibition of Nox4 expression in rat cells.

PROTOCOLS

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

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.

RT-PCR REAGENTS

Semi-quantitative RT-PCR may be performed to monitor Nox4 gene expression knockdown using RT-PCR Primer: Nox4 (r)-PR: sc-61887-PR (20 μ l, 479 bp). Annealing temperature for the primers should be 55-60° C and the extension temperature should be 68-72° C.

SELECT PRODUCT CITATIONS

1. Peshavariya, H.M., et al. 2007. Analysis of dihydroethidium fluorescence for the detection of intracellular and extracellular superoxide produced by NADPH oxidase. *Free Radic. Res.* 41: 699-712.
2. Zhang, H.S., et al. 2011. Akt/Nox2/NF κ B signaling pathway is involved in Tat-induced HIV-1 long terminal repeat (LTR) transactivation. *Arch. Biochem. Biophys.* 505: 266-272.
3. Nanduri, J., et al. 2013. Xanthine oxidase mediates hypoxia-inducible factor-2 α degradation by intermittent hypoxia. *PLoS ONE* 8: e75838.
4. Liu, X.H., et al. 2016. NADPH oxidase 4 contributes to connective tissue growth factor expression through Smad3-dependent signaling pathway. *Free Radic. Biol. Med.* 94: 174-184.
5. Lu, G., et al. 2017. H₂S inhibits Angiotensin II-induced atrial Kv1.5 upregulation by attenuating Nox4-mediated ROS generation during atrial fibrillation. *Biochem. Biophys. Res. Commun.* 483: 534-540.
6. Sun, Q., et al. 2017. Pharmacological inhibition of Nox4 ameliorates alcohol-induced liver injury in mice through improving oxidative stress and mitochondrial function. *Biochim. Biophys. Acta Gen. Subj.* 1861: 2912-2921.
7. Su, J., et al. 2018. Improvement of vascular dysfunction by argirein through inhibiting endothelial cell apoptosis associated with ET-1/Nox4 signal pathway in diabetic rats. *Sci. Rep.* 8: 12620.
8. Liu, X., et al. 2019. Nox4 and soluble epoxide hydrolase synergistically mediate homocysteine-induced inflammation in vascular smooth muscle cells. *Vascul. Pharmacol.* 120: 106544.
9. Yu, M.H., et al. 2019. SAA1 increases NOX4/ROS production to promote LPS-induced inflammation in vascular smooth muscle cells through activating p38MAPK/NF κ B pathway. *BMC Mol. Cell Biol.* 20: 15.

RESEARCH USE

For research use only, not for use in diagnostic procedures.