

Nox4 siRNA (m): sc-41587

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 Nox-4 in vascular oxidative stress response. The gene encoding human Nox4 maps to chromosome 11q14.3.

REFERENCES

1. Ushio-Fukai, M., et al. 1996. p22^{phox} is a critical component of the superoxide-generating NADH/NADPH oxidase system and regulates Angiotensin II-induced hypertrophy in vascular smooth muscle cells. *J. Biol. Chem.* 271: 23317-23321.
2. Archer, S.L., et al. 1999. O₂ sensing is preserved in mice lacking the gp91 phox subunit of NADPH oxidase. *Proc. Natl. Acad. Sci. USA* 96: 7944-7949.

CHROMOSOMAL LOCATION

Genetic locus: Nox4 (mouse) mapping to 7 D3.

PRODUCT

Nox4 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 Nox4 shRNA Plasmid (m): sc-41587-SH and Nox4 shRNA (m) Lentiviral Particles: sc-41587-V as alternate gene silencing products.

For independent verification of Nox4 (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-41587A, sc-41587B and sc-41587C.

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

Nox4 siRNA (m) is recommended for the inhibition of Nox4 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.

RT-PCR REAGENTS

Semi-quantitative RT-PCR may be performed to monitor Nox4 gene expression knockdown using RT-PCR Primer: Nox4 (m)-PR: sc-41587-PR (20 µl, 475 bp). Annealing temperature for the primers should be 55-60° C and the extension temperature should be 68-72° C.

SELECT PRODUCT CITATIONS

1. Xiao, Q., et al. 2009. Embryonic stem cell differentiation into smooth muscle cells is mediated by Nox4-produced H₂O₂. *Am. J. Physiol., Cell Physiol.* 296: C711-C723.
2. Zhao, R., et al. 2011. Involvement of NADPH oxidase in up-regulation of plasminogen activator inhibitor-1 and heat shock factor-1 in mouse embryo fibroblasts induced by oxidized LDL and in apolipoprotein E-deficient mice. *Free Radic. Res.* 45: 1013-1023.
3. Huang, W., et al. 2012. Simvastatin protects osteoblast against H₂O₂-induced oxidative damage via inhibiting the upregulation of Nox4. *Mol. Cell. Biochem.* 360: 71-77.
4. Galán, M., et al. 2014. Mechanism of endoplasmic reticulum stress-induced vascular endothelial dysfunction. *Biochim. Biophys. Acta* 1843: 1063-1075.
5. Ha, T.S., et al. 2016. Puromycin aminonucleoside increases podocyte permeability by modulating ZO-1 in an oxidative stress-dependent manner. *Exp. Cell Res.* 340: 139-149.
6. Lee, H.J., et al. 2017. Hydrogen sulfide inhibits high glucose-induced NADPH oxidase 4 expression and matrix increase by recruiting inducible nitric oxide synthase in kidney proximal tubular epithelial cells. *J. Biol. Chem.* 292: 5665-5675.
7. Min, S.Y., et al. 2018. Puromycin aminonucleoside triggers apoptosis in podocytes by inducing endoplasmic reticulum stress. *Kidney Res. Clin. Pract.* 37: 210-221.
8. Wu, Y.L., et al. 2019. Microcystin-LR promotes necroptosis in primary mouse hepatocytes by overproducing reactive oxygen species. *Toxicol. Appl. Pharmacol.* 377: 114626.

RESEARCH USE

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