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Nox5 siRNA (h): sc-45486

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. NADPH oxidase 5 (Nox5) is a homolog of the gp91 phox subunit of the phagocyte NADPH oxidase. Nox5 is expressed in lymphoid organs and testis and is distinguished from the other NADPH oxidases by its unique N-terminus, which contains three canonical EF-hands, Ca²⁺-binding domains. Upon heterologous expression, Nox5 generates superoxide in response to intracellular Ca²⁺ elevations.

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. Nisimoto, Y., et al. 1999. The p67^{phox} activation domain regulates electron flow from NADPH to flavin in flavocytochrome b₅₅₈. *J. Biol. Chem.* 274: 22999-23005.

CHROMOSOMAL LOCATION

Genetic locus: NOX5 (human) mapping to 15q23.

PRODUCT

Nox5 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 Nox5 shRNA Plasmid (h): sc-45486-SH and Nox5 shRNA (h) Lentiviral Particles: sc-45486-V as alternate gene silencing products.

For independent verification of Nox5 (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-45486A, sc-45486B and sc-45486C.

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

Nox5 siRNA (h) is recommended for the inhibition of Nox5 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

Nox5 (A-3): sc-518114 is recommended as a control antibody for monitoring of Nox5 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).

RT-PCR REAGENTS

Semi-quantitative RT-PCR may be performed to monitor Nox5 gene expression knockdown using RT-PCR Primer: Nox5 (h)-PR: sc-45486-PR (20 μl, 419 bp). Annealing temperature for the primers should be 55-60° C and the extension temperature should be 68-72° C.

SELECT PRODUCT CITATIONS

1. Montezano, A.C., et al. 2010. Nicotinamide adenine dinucleotide phosphate reduced oxidase 5 (Nox5) regulation by Angiotensin II and endothelin-1 is mediated via calcium/calmodulin-dependent, rac-1-independent pathways in human endothelial cells. *Circ. Res.* 106: 1363-1373.
2. Song, H.Y., et al. 2011. Nox2-based NADPH oxidase mediates HIV-1 Tat-induced up-regulation of VCAM-1/ICAM-1 and subsequent monocyte adhesion in human astrocytes. *Free Radic. Biol. Med.* 50: 576-584.
3. Manea, A., et al. 2012. Positive regulation of NADPH oxidase 5 by proinflammatory-related mechanisms in human aortic smooth muscle cells. *Free Radic. Biol. Med.* 52: 1497-1507.
4. Muzaffar, S., et al. 2012. NADPH oxidase 4 mediates upregulation of type 4 phosphodiesterases in human endothelial cells. *J. Cell. Physiol.* 227: 1941-1950.
5. Manea, A., et al. 2015. Human monocytes and macrophages express NADPH oxidase 5; a potential source of reactive oxygen species in atherosclerosis. *Biochem. Biophys. Res. Commun.* 461: 172-179.
6. Manuneechi Cholan, P., et al. 2018. TRAIL protects against endothelial dysfunction *in vivo* and inhibits Angiotensin-II-induced oxidative stress in vascular endothelial cells *in vitro*. *Free Radic. Biol. Med.* 126: 341-349.
7. Holterman, C.E., et al. 2019. Podocyte NADPH oxidase 5 promotes renal inflammation regulated by the Toll-like receptor pathway. *Antioxid. Redox Signal.* 30: 1817-1830.

RESEARCH USE

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