SANTA CRUZ BIOTECHNOLOGY, INC.

p22-phox siRNA (r): sc-61892

BACKGROUND

Mox1 and the glycoprotein gp91-phox are largely related proteins that are essential components of the NADPH oxidase. The superoxide-generating NADPH oxidase is present in phagocytes, neuroepithelial bodies, vascular smooth muscle cells and endothelial cells. It 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 O_2 sensors that initiate cellular signaling in response to hypoxic conditions.

REFERENCES

1. Henderson, L.M., et al. 1995. The arachidonate-activable, NADPH oxidase-associated H^+ channel. Evidence that gp91-phox functions as an essential part of the channel. J. Biol. Chem. 270: 5909-5916.
2. 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.
3. Nisimoto, Y., et al. 1999. The $p67^{phox}$ activation domain regulates electron flow from NADPH to flavin in flavocytochrome b_{558}. J. Biol. Chem. 274: 22999-23005.
4. Archer, S.L., et al. 1999. O_2 sensing is preserved in mice lacking the gp91 phox subunit of NADPH oxidase. Proc. Natl. Acad. Sci. USA 96: 7944-7949.
5. Yang, S., et al. 1999. Superoxide generation in transformed B-lymphocytes from patients with severe, malignant osteopetrosis. Mol. Cell. Biochem. 199: 15-24.
6. Meyer, J.W., et al. 1999. Identification of a functional leukocyte-type NADPH oxidase in human endothelial cells: a potential atherogenic source of reactive oxygen species. Endothelium 7: 11-22.
7. Suh, Y.A., et al. 1999. Cell transformation by the superoxide-generating oxidase Mox1. Nature 401: 79-82.

CHROMOSOMAL LOCATION

Genetic locus: Cyba (rat) mapping to 19q12.

PRODUCT

p22-phox 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 µM solution once resuspended using protocol below. Suitable for 50-100 transfections. Also see p22-phox shRNA Plasmid (r): sc-61892-SH and p22-phox shRNA (r) Lentiviral Particles: sc-61892-V as alternate gene silencing products.

For independent verification of p22-phox (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-61892A, sc-61892B and sc-61892C.

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

p22-phox siRNA (r) is recommended for the inhibition of p22-phox expression in rat 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

p22-phox (E-11): sc-271968 is recommended as a control antibody for monitoring of p22-phox 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 p22-phox gene expression knockdown using RT-PCR Primer: p22-phox (r)-PR: sc-61892-PR (20 µl, 415 bp). Annealing temperature for the primers should be 55-60° C and the extension temperature should be 68-72° C.

SELECT PRODUCT CITATIONS

1. Nunes Marsiglio-Librais, G., et al. 2020. Evidence for NADPH oxidase activation by GPR40 in pancreatic β-cells. Redox Rep. 25: 41-50.

RESEARCH USE

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