SANTA CRUZ BIOTECHNOLOGY, INC.

p22-phox siRNA (m): sc-36150

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.

CHROMOSOMAL LOCATION

Genetic locus: Cyba (mouse) mapping to 8 E1.

PRODUCT

p22-phox 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 p22-phox shRNA Plasmid (m): sc-36150-SH and p22-phox shRNA (m) Lentiviral Particles: sc-36150-V as alternate gene silencing products.

For independent verification of p22-phox (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-36150A, sc-36150B and sc-36150C.

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 (m) is recommended for the inhibition of p22-phox 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

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 (m)-PR: sc-36150-PR (20 µl, 416 bp). Annealing temperature for the primers should be 55-60° C and the extension temperature should be 68-72° C.

SELECT PRODUCT CITATIONS

1. Loane, D.J., et al. 2009. Activation of metabotropic glutamate receptor 5 modulates microglial reactivity and neurotoxicity by inhibiting NADPH oxidase. J. Biol. Chem. 284: 15629-15639.
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. Edderkaoui, M., et al. 2011. NADPH oxidase activation in pancreatic cancer cells is mediated through Akt-dependent up-regulation of p22-phox. J. Biol. Chem. 286: 7779-7787.
4. Zhao, R., et al. 2013. Regulatory role of NADPH oxidase in glycated LDL-induced upregulation of plasminogen activator inhibitor-1 and heat shock factor-1 in mouse embryo fibroblasts and diabetic mice. Free Radic. Biol. Med. 61: 18-25.
5. Luo, T., et al. 2013. Propofol limits microglial activation after experimental brain trauma through inhibition of nicotinamide adenine dinucleotide phosphate oxidase. Anesthesiology 119: 1370-1388.
6. Zhang, J., et al. 2014. Enhanced expression and activity of Nox2 and Nox4 in the macula densa in ANG II-induced hypertensive mice. Am. J. Physiol. Renal Physiol. 306: F344-F350.

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.