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gp91-phox siRNA (h2): sc-44302

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 O₂ sensors that initiate cellular signaling in response to hypoxic conditions. Mox1 and gp91 contain identical C-terminal sequence identity, yet they have distinct expression patterns. gp91-phox is expressed in eosinophils, neutrophils, monocytes and B-lymphocytes, whereas Mox1 is predominantly detected in the colon, and low expression is also detected in the uterus and prostate. Mox1 is also upregulated in vascular smooth-muscle cells in response to PDGF stimulation, which collectively indicates that Mox1 may function analogously to gp91-phox, yet regulate the NADPH superoxide production in non-phagocytic cells.

REFERENCES

- 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.
- 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.
- Suh, Y.A., et al. 1999. Cell transformation by the superoxide-generating oxidase Mox1. *Nature* 401: 79-82.
- 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: CYBB (human) mapping to Xp11.4.

PRODUCT

gp91-phox siRNA (h2) 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 gp91-phox shRNA Plasmid (h2): sc-44302-SH and gp91-phox shRNA (h2) Lentiviral Particles: sc-44302-V as alternate gene silencing products.

For independent verification of gp91-phox (h2) gene silencing results, we also provide the individual siRNA duplex components. Each is available as 3.3 nmol of lyophilized siRNA. These include: sc-44302A, sc-44302B and sc-44302C.

RESEARCH USE

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

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

gp91-phox siRNA (h2) is recommended for the inhibition of gp91-phox 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

gp91-phox (54.1): sc-130543 is recommended as a control antibody for monitoring of gp91-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 BPHRP: sc-516102 or m-IgGκ BPHRP (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 BPFITC: sc-516140 or m-IgGκ BPE: 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 gp91-phox gene expression knockdown using RT-PCR Primer: gp91-phox (h2)-PR: sc-44302-PR (20 μl, 500 bp). Annealing temperature for the primers should be 55-60° C and the extension temperature should be 68-72° C.

PROTOCOLS

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