



p67-phox siRNA (h): sc-36163

BACKGROUND

The hereditary disease chronic granulomatous disease (CGD) has been linked to mutations in p47-phox and p67-phox. The cytosolic proteins p47-phox and p67-phox, also designated neutrophil cytosol factor (NCF)1 and NCF2, respectively, are required for activation of the superoxide-producing NADPH oxidase in neutrophils and other phagocytic cells. During activation of the NADPH oxidase, p47-phox and p67-phox migrate to the plasma membrane where they associate with cytochrome b558 and the small G protein Rac to form the functional enzyme complex. Both p47-phox and p67-phox contain two Src homology 3 (SH3) domains. The C-terminal SH3 domain of p67-phox has been shown to interact with the proline rich domain of p47-phox, suggesting that p47-phox may facilitate the transport of p67-phox to the membrane.

REFERENCES

1. Lomax, K.J., et al. 1989. Recombinant 47-kilodalton cytosol factor restores NADPH oxidase in chronic granulomatous disease. *Science* 245: 409-412.
2. Heyworth, P.G., et al. 1991. Neutrophil nicotinamide adenine dinucleotide phosphate oxidase assembly. Translocation of p47-phox and p67-phox requires interaction between p47-phox and cytochrome b558. *J. Clin. Invest.* 87: 352-356.
3. Kenney, R.T., et al. 1993. Characterization of the p67-phox gene: genomic organization and restriction fragment length polymorphism analysis for prenatal diagnosis in chronic granulomatous disease. *Blood* 82: 3739-3744.
4. Finan, P., et al. 1994. An SH3 domain and proline-rich sequence mediate an interaction between two components of the phagocyte NADPH oxidase complex. *J. Biol. Chem.* 269: 13752-13755.

CHROMOSOMAL LOCATION

Genetic locus: NCF2 (human) mapping to 1q25.3.

PRODUCT

p67-phox 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 μ M solution once resuspended using protocol below. Suitable for 50-100 transfections. Also see p67-phox shRNA Plasmid (h): sc-36163-SH and p67-phox shRNA (h) Lentiviral Particles: sc-36163-V as alternate gene silencing products.

For independent verification of p67-phox (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-36163A, sc-36163B and sc-36163C.

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 μ l of the RNase-free water provided. Resuspension of the siRNA duplex in 330 μ l of RNase-free water makes a 10 μ M solution in a 10 μ M Tris-HCl, pH 8.0, 20 mM NaCl, 1 mM EDTA buffered solution.

APPLICATIONS

p67-phox siRNA (h) is recommended for the inhibition of p67-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 μ M in 66 μ l. 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

p67-phox (D-6): sc-374510 is recommended as a control antibody for monitoring of p67-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-IgG κ BP-HRP: sc-516102 or m-IgG κ 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-IgG κ BP-FITC: sc-516140 or m-IgG κ 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 p67-phox gene expression knockdown using RT-PCR Primer: p67-phox (h)-PR: sc-36163-PR (20 μ l, 483 bp). Annealing temperature for the primers should be 55-60° C and the extension temperature should be 68-72° C.

SELECT PRODUCT CITATIONS

1. Lee, W.J., et al. 2014. N ϵ -carboxymethyllysine-mediated endoplasmic reticulum stress promotes endothelial cell injury through Nox4/MKP-3 interaction. *Free Radic. Biol. Med.* 74: 294-306.
2. Vo, T.T.T., et al. 2021. Carbon monoxide-releasing molecule-2 ameliorates particulate matter-induced aorta inflammation via toll-like receptor/ NADPH oxidase/ROS/NF- κ B/IL-6 inhibition. *Oxid. Med. Cell. Longev.* 2021: 2855042.
3. Belambri, S.A., et al. 2022. Impaired p47phox phosphorylation in neutrophils from patients with p67phox-deficient chronic granulomatous disease. *Blood* 139: 2512-2522.

RESEARCH USE

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