

p40-phox siRNA (m): sc-36156

BACKGROUND

Nicotinamide adenine dinucleotide phosphate (NADPH)-oxidase is a multi-meric enzyme system that mediates electron transport from NADPH in the cytoplasm to molecular oxygen in the phagosome, thereby generating reactive oxidant intermediates. Upon neutrophil stimulation, NADPH-oxidase and other cytosolic elements localize to the cell membrane from the cytosol to form a complex which produces phagocytic oxygen radicals. There are a number of cytosolic proteins that are involved in NADPH-oxidase activation/deactivation, including p47-phox, p67-phox, p40-phox and the small GTP-binding protein, Rac. Activation of NADPH oxidase is accompanied by the phosphorylation of cytosolic components p40-phox, p47-phox and p67-phox. The PKC consensus phosphorylation sites Thr 154 and Ser 315 in p40-phox are phosphorylated during activation of NADPH oxidase. p40-phox can promote oxidase activation by increasing the affinity of p47-phox for NADPH-oxidase. However, p40-phox appears to downregulate oxidase function as well, by competing with an SH3 domain interaction between other essential oxidase components.

REFERENCES

1. Sathiyamoorthy, M., et al. 1997. p40-phox down-regulates NADPH oxidase activity through interactions with its SH3 domain. *J. Biol. Chem.* 272: 9141-9146.
2. Bouin, A.P., et al. 1998. p40-phox is phosphorylated on threonine 154 and serine 315 during activation of the phagocyte NADPH oxidase. Implication of a protein kinase c-type kinase in the phosphorylation process. *J. Biol. Chem.* 273: 30097-30103.
3. Someya, A., et al. 1999. Phosphorylation of p40-phox during activation of neutrophil NADPH oxidase. *J. Leukoc. Biol.* 66: 851-887.
4. Cross, A.R. 2000. p40-phox participates in the activation of NADPH oxidase by increasing the affinity of p47-phox for flavocytochrome b(558). *Biochem. J.* 349: 113-117.
5. Wientjes, F.B., et al. 2001. The NADPH oxidase components p47-phox and p40-phox bind to moesin through their PX domain. *Biochem. Biophys. Res. Commun.* 289: 382-388.
6. Online Mendelian Inheritance in Man, OMIM[™]. 2001. Johns Hopkins University, Baltimore, MD. MIM Number: 601488. World Wide Web URL: <http://www.ncbi.nlm.nih.gov/omim/>

CHROMOSOMAL LOCATION

Genetic locus: Ncf4 (mouse) mapping to 15 E1.

PRODUCT

p40-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 μ M solution once resuspended using protocol below. Suitable for 50-100 transfections. Also see p40-phox shRNA Plasmid (m): sc-36156-SH and p40-phox shRNA (m) Lentiviral Particles: sc-36156-V as alternate gene silencing products.

For independent verification of p40-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-36156A, sc-36156B and sc-36156C.

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

p40-phox siRNA (m) is recommended for the inhibition of p40-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 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

p40-phox (D-8): sc-48388 is recommended as a control antibody for monitoring of p40-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 p40-phox gene expression knockdown using RT-PCR Primer: p40-phox (m)-PR: sc-36156-PR (20 μ l, 579 bp). Annealing temperature for the primers should be 55-60° C and the extension temperature should be 68-72° C.

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.