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SANTA CRUZ BIOTECHNOLOGY, INC.

p47-phox siRNA (r): sc-45918

BACKGROUND

The heredity chronic granulomatous disease (CGF) 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 faciliate the transport of p67-phox to the membrane.

CHROMOSOMAL LOCATION

Genetic locus: Ncf1 (rat) mapping to 12q12.

PRODUCT

p47-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 p47-phox shRNA Plasmid (r): sc-45918-SH and p47-phox shRNA (r) Lentiviral Particles: sc-45918-V as alternate gene silencing products.

For independent verification of p47-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-45918A, sc-45918B and sc-45918C.

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

p47-phox siRNA (r) is recommended for the inhibition of p47-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

p47-phox (D-10): sc-17845 is recommended as a control antibody for monitoring of p47-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 p47-phox gene expression knockdown using RT-PCR Primer: p47-phox (r)-PR: sc-45918-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. Wei, Y., et al. 2006. Angiotensin II-induced NADPH oxidase activation impairs Insulin signaling in skeletal muscle cells. J. Biol. Chem. 281: 35137-35146.
2. Liu, S., et al. 2007. Glucose down-regulation of cGMP-dependent protein kinase I expression in vascular smooth muscle cells involves NAD(P)H oxidase-derived reactive oxygen species. Free Radic. Biol. Med. 42: 852-863.
3. Shen, E., et al. 2009. Rac1 is required for cardiomyocyte apoptosis during hyperglycemia. Diabetes 58: 2386-2395.
4. Yeligar, S.M., et al. 2009. Ethanol augments RANTES/CCL5 expression in rat liver sinusoidal endothelial cells and human endothelial cells via activation of NFκB, HIF-1 α, and AP-1. J. Immunol. 183: 5964-5976.
5. Subasinghe, W., et al. 2011. Phagocyte-like NADPH oxidase promotes cytokine-induced mitochondrial dysfunction in pancreatic β-cells: evidence for regulation by Rac1. Am. J. Physiol. Regul. Integr. Comp. Physiol. 300: R12-R20.
6. Syed, I., et al. 2011. Phagocyte-like NADPH oxidase generates ROS in INS 832/13 cells and rat islets: role of protein prenylation. Am. J. Physiol. Regul. Integr. Comp. Physiol. 300: R756-R762.
7. Wang, P., et al. 2013. Hydrogen peroxide-mediated oxidative stress and collagen synthesis in cardiac fibroblasts: blockade by tanshinone IIA. J. Ethnopharmacol. 145: 152-161.

RESEARCH USE

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