SANTA CRUZ BIOTECHNOLOGY, INC.

p47phox siRNA (r): sc-45918

BACKGROUND

The heredity chronic granulomatous disease (CGF) has been linked to mutations in p47phox and p67-phox. The cytosolic proteins p47phox 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, p47phox 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 p47phox 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 p47phox, suggesting that p47phox may faciliate the transport of p67-phox to the membrane.

CHROMOSOMAL LOCATION

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

PRODUCT

p47phox 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 p47phox shRNA Plasmid (r): sc-45918-SH and p47phox shRNA (r) Lentiviral Particles: sc-45918-V as alternate gene silencing products.

For independent verification of p47phox (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

p47phox siRNA (r) is recommended for the inhibition of p47phox 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

p47phox (D-10): sc-17845 is recommended as a control antibody for monitoring of p47phox 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).

RT-PCR REAGENTS

Semi-quantitative RT-PCR may be performed to monitor p47phox gene expression knockdown using RT-PCR Primer: p47phox (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-1α, and AP-1. J. Immunol. 183: 5964-5976.
5. Li, Y. and Wang, S. 2010. Glycated albumin activates NADPH oxidase in rat mesangial cells through up-regulation of p47phox. Biochem. Biophys. Res. Commun. 397: 5-11.
6. 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.
7. 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.
8. 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.
9. Contreras-Ferrat, A., et al. 2014. Insulin elicits a Ros-activated and an IP_3-dependent Ca^{2+} release, which both impinge on GLUT4 translocation. J. Cell Sci. 127: 1911-1923.

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.