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### SZABO-SCANDIC HandelsgmbH

Quellenstraße 110, A-1100 Wien

T. +43(0)1 489 3961-0

F. +43(0)1 489 3961-7

[mail@szabo-scandic.com](mailto:mail@szabo-scandic.com)

[www.szabo-scandic.com](http://www.szabo-scandic.com)

[linkedin.com/company/szaboscandic](https://www.linkedin.com/company/szaboscandic) 

# p47-phox shRNA (r) Lentiviral Particles: sc-45918-V

## BACKGROUND

The hereditary 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 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: Ncf1 (rat) mapping to 12q12.

## PRODUCT

p47-phox shRNA (r) Lentiviral Particles is a pool of concentrated, transduction-ready viral particles containing 3 target-specific constructs that encode 19-25 nt (plus hairpin) shRNA designed to knock down gene expression. Each vial contains 200  $\mu$ l frozen stock containing  $1.0 \times 10^6$  infectious units of virus (IFU) in Dulbecco's Modified Eagle's Medium with 25 mM HEPES pH 7.3. Suitable for 10-20 transductions. Also see p47-phox siRNA (r): sc-45918 and p47-phox shRNA Plasmid (r): sc-45918-SH as alternate gene silencing products.

## RESEARCH USE

The purchase of this product conveys to the buyer the nontransferable right to use the purchased amount of the product and all replicates and derivatives for research purposes conducted by the buyer in his laboratory only (whether the buyer is an academic or for-profit entity). The buyer cannot sell or otherwise transfer (a) this product (b) its components or (c) materials made using this product or its components to a third party, or otherwise use this product or its components or materials made using this product or its components for Commercial Purposes.

## APPLICATIONS

p47-phox shRNA (r) Lentiviral Particles is recommended for the inhibition of p47-phox expression in rat cells.

## SUPPORT REAGENTS

Control shRNA Lentiviral Particles: sc-108080. Available as 200  $\mu$ l frozen viral stock containing  $1.0 \times 10^6$  infectious units of virus (IFU); contains an shRNA construct encoding a scrambled sequence that will not lead to the specific degradation of any known cellular mRNA.

## 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 (secondary) reagents are recommended: 1) Western Blotting: use goat anti-mouse IgG-HRP: sc-2005 (dilution range: 1:2000-1:32,000) or Cruz Marker™ compatible goat anti-mouse IgG-HRP: sc-2031 (dilution range: 1:2000-1:5000), Cruz Marker™ Molecular Weight Standards: sc-2035, TBS Blotto A Blocking Reagent: sc-2333 and Western Blotting Luminol Reagent: sc-2048. 2) Immunofluorescence: use goat anti-mouse IgG-FITC: sc-2010 (dilution range: 1:100-1:400) or goat anti-mouse IgG-TR: sc-2781 (dilution range: 1:100-1:400) with UltraCruz™ Mounting Medium: sc-24941.

## 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  $\mu$ l, 415 bp). Annealing temperature for the primers should be 55-60° C and the extension temperature should be 68-72° C.

## BIOSAFETY

Lentiviral particles can be employed in standard Biosafety Level 2 tissue culture facilities (and should be treated with the same level of caution as with any other potentially infectious reagent). Lentiviral particles are replication-incompetent and are designed to self-inactivate after transduction and integration of shRNA constructs into genomic DNA of target cells.

## STORAGE

Store lentiviral particles at -80° C. Stable for at least one year from the date of shipment. Once thawed, particles can be stored at 4° C for up to one week. Avoid repeated freeze thaw cycles.

## PROTOCOLS

See our web site at [www.scbt.com](http://www.scbt.com) or our catalog for detailed protocols and support products.