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p47 siRNA (m): sc-151963

BACKGROUND

p47, also known as NSFL1C, UBX1, UBXD10 or UBXN2C, is a 370 amino acid protein that localizes to both the nucleus and the golgi apparatus (specifically to golgi stacks) and contains one SEP domain and one UBX domain. Functioning as part of a ternary complex with VCP (a protein involved in the heterotypic fusion of transport vesicles with their target membranes) and Syntaxin 5, p47 interacts with and reduces the ATPase activity of VCP and is required for the fragmentation of Golgi stacks during mitosis and for subsequent re-assembly of golgi stacks after mitosis. p47 is subject to phosphorylation during mitosis, which inhibits p47-Golgi interaction and is, therefore, required for proper Golgi stack formation and cisternal regrowth. Human p47 shares 89% sequence identity with its mouse counterpart, suggesting a conserved role between species. Multiple isoforms of p47 exist due to alternative splicing events.

REFERENCES

1. Kondo, H., et al. 1997. p47 is a cofactor for p97-mediated membrane fusion. *Nature* 388: 75-78.
2. Rabouille, C., et al. 1998. Syntaxin 5 is a common component of the NSF- and p97-mediated reassembly pathways of Golgi cisternae from mitotic Golgi fragments *in vitro*. *Cell* 92: 603-610.
3. Ye, Y., et al. 2001. The AAA ATPase Cdc48/p97 and its partners transport proteins from the ER into the cytosol. *Nature* 414: 652-656.
4. Meyer, H.H., et al. 2002. Direct binding of ubiquitin conjugates by the mammalian p97 adaptor complexes, p47 and Ufd1-Npl4. *EMBO J.* 21: 5645-5652.

CHROMOSOMAL LOCATION

Genetic locus: Nsf1c (mouse) mapping to 2 G3.

PRODUCT

p47 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 p47 shRNA Plasmid (m): sc-151963-SH and p47 shRNA (m) Lentiviral Particles: sc-151963-V as alternate gene silencing products.

For independent verification of p47 (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-151963A, sc-151963B and sc-151963C.

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

p47 siRNA (m) is recommended for the inhibition of p47 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

p47 (D-9): sc-365215 is recommended as a control antibody for monitoring of p47 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 p47 gene expression knockdown using RT-PCR Primer: p47 (m)-PR: sc-151963-PR (20 μ l). Annealing temperature for the primers should be 55-60° C and the extension temperature should be 68-72° C.

SELECT PRODUCT CITATIONS

1. Zhao, Y., et al. 2008. Inhibition of NADPH oxidase activity promotes differentiation of B16 melanoma cells. *Oncol. Rep.* 19: 1225-1230.
2. Li, J.T., et al. 2016. Subanesthetic isoflurane relieves Zymosan-induced neutrophil inflammatory response by targeting NMDA glutamate receptor and Toll-like receptor 2 signaling. *Oncotarget* 7: 31772-31789.

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.