

NP60 siRNA (h): sc-93243

BACKGROUND

MAP (mitogen-activated protein) kinases play a significant role in many biological processes, including cell adhesion and spreading, cell differentiation and apoptosis. Via their catalytic activity, MAP kinases p38 α , p38 β and p38 γ are involved in a variety of events throughout the cell, including signal transduction pathways, cytokine production and cell proliferation and differentiation. The p38 proteins are subject to phosphorylation on Thr and Tyr residues, an event which is thought to activate the phosphorylated protein. NP60 (nuclear protein of 60 kDa), also known as Glyoxylate reductase 1 homolog, 3-hydroxyisobutyrate dehydrogenase-like protein and Cytokine-like nuclear factor N-PAC, is a 553 amino acid nuclear protein that regulates the phosphorylation and activation of p38 α in response to stress. There are five isoforms of NP60 that are produced as a result of alternative splicing events.

REFERENCES

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2. Roux, P.P. and Blenis, J. 2004. ERK and p38 MAPK-activated protein kinases: a family of protein kinases with diverse biological functions. *Microbiol. Mol. Biol. Rev.* 68: 320-344.
3. Mittelstadt, P.R., et al. 2005. Activating p38 MAPK: new tricks for an old kinase. *Cell Cycle* 4: 1189-1192.
4. Kang, Y.J., et al. 2006. Multiple activation mechanisms of p38 α mitogen-activated protein kinase. *J. Biol. Chem.* 281: 26225-26234.
5. Fu, J., et al. 2006. Nuclear protein NP60 regulates p38 MAPK activity. *J. Cell Sci.* 119: 115-123.
6. Ashwell, J.D. 2006. The many paths to p38 mitogen-activated protein kinase activation in the immune system. *Nat. Rev. Immunol.* 6: 532-540.

CHROMOSOMAL LOCATION

Genetic locus: GLYR1 (human) mapping to 16p13.3.

PRODUCT

NP60 siRNA (h) 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 NP60 shRNA Plasmid (h): sc-93243-SH and NP60 shRNA (h) Lentiviral Particles: sc-93243-V as alternate gene silencing products.

For independent verification of NP60 (h) gene silencing results, we also provide the individual siRNA duplex components. Each is available as 3.3 nmol of lyophilized siRNA. These include: sc-93243A, sc-93243B and sc-93243C.

PROTOCOLS

See our web site at www.scbt.com for detailed protocols and support products.

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

NP60 siRNA (h) is recommended for the inhibition of NP60 expression in human 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

NP60 (D-10): sc-390601 is recommended as a control antibody for monitoring of NP60 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 NP60 gene expression knockdown using RT-PCR Primer: NP60 (h)-PR: sc-93243-PR (20 μ l). 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.