

PAC-1 siRNA (h): sc-39004

BACKGROUND

Mitogen-activated protein (MAP) kinases are a large class of proteins involved in signal transduction pathways that are activated by a range of stimuli and mediate a number of physiological and pathological changes in the cell. Dual specificity phosphatases (DSPs) are a subclass of the protein tyrosine phosphatase (PTP) gene superfamily, which are selective for dephosphorylating critical phosphothreonine and phosphotyrosine residues within MAP kinases. DSP gene expression is induced by a host of growth factors and/or cellular stresses, thereby negatively regulating MAP kinase superfamily members including MAPK/ERK, SAPK/JNK and p38. The members of the dual-specificity phosphatase protein family include MKP-1/CL100 (3CH134), VHR, PAC-1, MKP-2, hVH-3 (B23), hVH-5, MKP-3, MKP-X, and MKP-4. Human PAC-1 maps to chromosome 2q11.2 and encodes a 314 amino acid, mitogen-induced protein.

REFERENCES

1. Keyse, S.M. 1995. An emerging family of dual specificity MAP kinase phosphatases. *Biochim. Biophys. Acta* 1265: 152-160.
2. Grumont, R.J., et al. 1996. Activation of the mitogen-activated protein kinase pathway induces transcription of the PAC-1 phosphatase gene. *Mol. Cell. Biol.* 36: 2913-2921.
3. Muda, M., et al. 1997. Molecular cloning and functional characterization of a novel mitogen-activated protein kinase phosphatase, MKP-4. *J. Biol. Chem.* 272: 5141-5151.
4. Sun, H. 1998. Functional studies of dual-specificity phosphatases. *Methods Mol. Biol.* 84: 307-318.

CHROMOSOMAL LOCATION

Genetic locus: DUSP2 (human) mapping to 2q11.2.

PRODUCT

PAC-1 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 PAC-1 shRNA Plasmid (h): sc-39004-SH and PAC-1 shRNA (h) Lentiviral Particles: sc-39004-V as alternate gene silencing products.

For independent verification of PAC-1 (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-39004A, sc-39004B and sc-39004C.

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

PAC-1 siRNA (h) is recommended for the inhibition of PAC-1 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

PAC-1 (4021): sc-32776 is recommended as a control antibody for monitoring of PAC-1 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 PAC-1 gene expression knockdown using RT-PCR Primer: PAC-1 (h)-PR: sc-39004-PR (20 μ l, 564 bp). Annealing temperature for the primers should be 55-60° C and the extension temperature should be 68-72° C.

SELECT PRODUCT CITATIONS

1. Kim, M.J., et al. 2010. AMP-activated protein kinase antagonizes pro-apoptotic extracellular signal-regulated kinase activation by inducing dual-specificity protein phosphatases in response to glucose deprivation in HCT116 carcinoma. *J. Biol. Chem.* 285: 14617-14627.
2. Radpour, R., et al. 2021. Epigenetic silencing of immune-checkpoint receptors in bone marrow-infiltrating T cells in acute myeloid leukemia. *Front. Oncol.* 11: 663406.

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.