SANTA CRUZ BIOTECHNOLOGY, INC.

PYST2 siRNA (h): sc-61427

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. PYST2 inactivates MAPK/ERK, thereby regulating the MAP kinase signaling pathway. PYST2 is overexpressed in patients with acute myelogenous leukemia (AML).

REFERENCES

1. Keyse, S.M. 1995. An emerging family of dual specificity MAP kinase phosphatases. Biochim. Biophys. Acta 1265: 152-160.
2. Sun, H. 1998. Functional studies of dual-specificity phosphatases. Methods Mol. Biol. 84: 307-18.
3. Dowd, S., Sneddon, A.A. and Keyse, S.M. 1999. Isolation of the human genes encoding the PYST1 and PYST2 phosphatases: characterisation of PYST2 as a cytosolic dual-specificity MAP kinase phosphatase and its catalytic activation by both MAP and SAP kinases. J. Cell Sci. 111: 3389-3399.
4. Camps, M., Nichols, A. and Arkinstall, S. 2000. Dual specificity phosphatases: a gene family for control of MAP kinase function. FASEB J. 14: 6-16.
5. Levy-Nissenbaum, O., Sagi-Assif, O., Raanani, P., Avigdor, A., Ben-Bassat, I. and Witz, I.P. 2003. cDNA microarray analysis reveals an overexpression of the dual-specificity MAPK phosphatase PYST2 in acute leukemia. Methods Enzymol. 366: 103-113.
6. Levy-Nissenbaum, O., Sagi-Assif, O., Raanani, P., Avigdor, A., Ben-Bassat, I. and Witz, I.P. 2003. Overexpression of the dual-specificity MAPK phosphatase PYST2 in acute leukemia. Cancer Lett. 199: 185-192.
7. Levy-Nissenbaum, O., Sagi-Assif, O. and Witz, I.P. 2003. Characterization of the dual-specificity phosphatase PYST2 and its transcripts. Genes Chromosomes Cancer 39: 37-47.

CHROMOSOMAL LOCATION

Genetic locus: DUSP7 (human) mapping to 3p21.2.

PRODUCT

PYST2 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 µM solution once resuspended using protocol below. Suitable for 50-100 transfections. Also see PYST2 shRNA Plasmid (h): sc-61427-SH and PYST2 shRNA (h) Lentiviral Particles: sc-61427-V as alternate gene silencing products.

For independent verification of PYST2 (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-61427A, sc-61427B and sc-61427C.

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

PYST2 siRNA (h) is recommended for the inhibition of PYST2 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 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

PYST2 (D-8): sc-377106 is recommended as a control antibody for monitoring of PYST2 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-IgGκ BP-HRP: sc-516102 or m-IgGκ 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-IgGκ BP-FITC: sc-516140 or m-IgGκ 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 PYST2 gene expression knockdown using RT-PCR Primer: PYST2 (h)-PR: sc-61427-PR (20 µl). 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.

PROTOCOLS

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