

3pK siRNA (h): sc-39105

BACKGROUND

The MAPKAP kinases (for MAP kinase activated protein kinases) are a group of MAP kinase substrates which are themselves kinases. In response to activation, the MAP kinases phosphorylate downstream components on a consensus Pro-X-Ser/Thr-Pro motif. Several kinases that contain this motif have been identified and serve as substrates for the ERK and p38 MAP kinases. These include the serine/threonine kinases Rsk-1 (also designated MAPKAP kinase-1), Rsk-2 and Rsk-3, which are phosphorylated by ERK1 and ERK2. Similarly, p38 phosphorylates and activates the serine/threonine kinases MAPKAP kinase-2 and MAPKAP kinase-3 (also designated 3pK). The serine/threonine kinases Mnk1 and Mnk2 are substrates for both ERK and p38 MAP kinases.

REFERENCES

1. Sturgill, T.W., et al. 1988. Insulin-stimulated MAP2 kinase phosphorylates and activates ribosomal protein S6 kinase II. *Nature* 334: 715-718.
2. Stokoe, D., et al. 1992. MAPKAP kinase-2: a novel protein kinase activated by mitogen-activated protein kinase. *EMBO J.* 11: 3985-3994.
3. Davis, R.J. 1993. The mitogen-activated protein kinase signal transduction pathway. *J. Biol. Chem.* 268: 14553-14556.
4. Zhao, Y., et al. 1995. Rsk-3 encodes a novel pp90Rsk isoform with a unique N-terminal sequence: growth factor stimulated kinase function and nuclear translocation. *Mol. Cell. Biol.* 15: 4353-4363.
5. Sithanandam, G., et al. 1996. 3pK, a new mitogen-activated protein kinase-activated protein kinase located in the small cell lung cancer tumor suppressor gene region. *Mol. Cell. Biol.* 16: 868-876.
6. McLaughlin, M.M., et al. 1996. Identification of mitogen-activated protein (MAP) kinase-activated protein kinase-3, a novel substrate of CSBP p38 MAP kinase. *J. Biol. Chem.* 271: 8488-8492.
7. Waskiewicz, A.J., et al. 1997. Mitogen-activated protein kinases activate the serine/threonine kinases Mnk1 and Mnk2. *EMBO J.* 16: 1090-1092.
8. Fukunaga, R., et al. 1997. Mnk1, a new MAP kinase-activated protein kinase, isolated by a novel expression screening method for identifying protein kinase substrates. *EMBO J.* 16: 1921-1933.

CHROMOSOMAL LOCATION

Genetic locus: IFNGR2 (human) mapping to 3p21.2.

PRODUCT

3pK 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 3pK shRNA Plasmid (h): sc-39105-SH and 3pK shRNA (h) Lentiviral Particles: sc-39105-V as alternate gene silencing products.

For independent verification of 3pK (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-39105A, sc-39105B and sc-39105C.

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

3pK siRNA (h) is recommended for the inhibition of 3pK 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

3pK (C-12): sc-376743 is recommended as a control antibody for monitoring of 3pK 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 3pK gene expression knockdown using RT-PCR Primer: 3pK (h)-PR: sc-39105-PR (20 μ l, 455 bp). 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.