



PRAK siRNA (m): sc-36311

BACKGROUND

PRAK (p38-regulated/activated kinase), also referred to as mitogen-activated protein kinase (MAPK)-activated protein kinase (MAPKAPK)-5, is a ubiquitously expressed serine/threonine kinase regulated by p38 α and p38 β MAP kinases. Activated JNK, p38 γ or p38 δ are unable to induce phosphorylation of PRAK *in vitro*. Phosphorylation of PRAK occurs *in vivo* in response to p38 activation by stress-related extracellular stimuli including UV light, oxidation and proinflammatory cytokines. Two other substrates for p38, MAPKAPK-2 and MAPKAPK-3/3pK, share approximately 45% sequence homology with PRAK including the phosphorylation motif recognized by p38, Lys-X-Thr-Pro. Activated PRAK has been shown to specifically phosphorylate HSP 27 *in vitro*, suggesting that the protein may play a role in stress-induced small heat shock protein phosphorylation *in vivo*.

REFERENCES

1. Stokoe, D., et al. 1992. MAPKAP kinase-2; a novel protein kinase activated by mitogen-activated protein kinase. *EMBO J.* 11: 3985-3994.
2. Raingeaud, J., et al. 1995. Pro-inflammatory cytokines and environmental stress cause p38 mitogen-activated protein kinase activation by dual phosphorylation on tyrosine and threonine. *J. Biol. Chem.* 270: 7420-7426.
3. 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.
4. New, L., et al. 1998. PRAK, a novel protein kinase regulated by the p38 MAP kinase. *EMBO J.* 17: 3372-3384.

CHROMOSOMAL LOCATION

Genetic locus: Mapkapk5 (mouse) mapping to 5 F.

PRODUCT

PRAK 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 PRAK shRNA Plasmid (m): sc-36311-SH and PRAK shRNA (m) Lentiviral Particles: sc-36311-V as alternate gene silencing products.

For independent verification of PRAK (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-36311A, sc-36311B and sc-36311C.

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

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

PRAK (A-7): sc-46667 is recommended as a control antibody for monitoring of PRAK 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 PRAK gene expression knockdown using RT-PCR Primer: PRAK (m)-PR: sc-36311-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. Ronkina, N., et al. 2015. Comparative analysis of two gene-targeting approaches challenges the tumor-suppressive role of the protein kinase MK5/PRAK. *PLoS ONE* 10: e0136138.

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.