



MARK3 siRNA (m): sc-60087

BACKGROUND

MARK3 (MAP/microtubule affinity-regulating kinase 3), also known as C-TAK1 (Cdc25C-associated protein kinase 1), EMK-2 (ELKL motif kinase 2), protein kinase STK10 or serine/threonine-protein kinase p78, is a 753 amino acid peripheral membrane protein that phosphorylates Cdc25C on Serine 216 and is ubiquitously expressed in various human tissue and cell lines. Existing as six alternatively spliced isoforms, MARK3 belongs to the Ser/Thr protein kinase family and the protein kinase superfamily. MARK3 has been suggested to mediate the binding of the 14-3-3 proteins through its kinase activity and acts as a negative regulator of mitosis. The gene encoding MARK3 maps to human chromosome 14q32.3 and mouse chromosome 12 F1.

REFERENCES

- Ogg, S., et al. 1994. Purification of a serine kinase that associates with and phosphorylates human Cdc25C on serine 216. *J. Biol. Chem.* 269: 30461-30469.
- Sanchez, Y., et al. 1997. Conservation of the Chk1 checkpoint pathway in mammals: linkage of DNA damage to Cdk regulation through Cdc25. *Science* 277: 1497-1501.
- Peng, C.Y., et al. 1997. Mitotic and G₂ checkpoint control: regulation of 14-3-3 protein binding by phosphorylation of Cdc25C on serine-216. *Science* 277: 1501-1505.
- Peng, C.Y., et al. 1998. C-TAK1 protein kinase phosphorylates human Cdc25C on serine 216 and promotes 14-3-3 protein binding. *Cell Growth Differ.* 9: 197-208.
- Dalal, S.N., et al. 1999. Cytoplasmic localization of human Cdc25C during interphase requires an intact 14-3-3 binding site. *Mol. Cell. Biol.* 19: 4465-4479.
- Chen, L., et al. 1999. Association of Chk1 with 14-3-3 proteins is stimulated by DNA damage. *Genes Dev.* 13: 675-685.

CHROMOSOMAL LOCATION

Genetic locus: Mark3 (mouse) mapping to 12 F1.

PRODUCT

MARK3 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 MARK3 shRNA Plasmid (m): sc-60087-SH and MARK3 shRNA (m) Lentiviral Particles: sc-60087-V as alternate gene silencing products.

For independent verification of MARK3 (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-60087A, sc-60087B and sc-60087C.

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

MARK3 siRNA (m) is recommended for the inhibition of MARK3 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

MARK3 (G-11): sc-365461 is recommended as a control antibody for monitoring of MARK3 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 MARK3 gene expression knockdown using RT-PCR Primer: MARK3 (m)-PR: sc-60087-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.