

# CaMKK $\beta$ siRNA (m): sc-38956

## BACKGROUND

The Ca<sup>2+</sup>/calmodulin-dependent protein kinases (CaM kinases) are a structurally related subfamily of serine/threonine kinases that includes CaMKI, CaMKII and CaMKIV. CaMKI and CaMKIV are stimulated by Ca<sup>2+</sup> and CaM, but phosphorylation by a CaMK is also required for full activation. CaMKK $\alpha$  and CaMKK $\beta$  function to activate CaMKI through the specific phosphorylation of the regulatory threonine residue at position 177. CaMKK $\beta$  is also capable of phosphorylating CaMKIV on threonine residue 200.

## REFERENCES

1. Kitani, T., et al. 1994. cDNA cloning and expression of human calmodulin-dependent protein kinase IV. *J. Biochem.* 115: 637-640.
2. Haribabu, B., et al. 1995. Human calcium-calmodulin dependent protein kinase I: cDNA cloning, domain structure and activation by phosphorylation at threonine-177 by calcium-calmodulin dependent protein kinase I kinase. *EMBO J.* 14: 3679-3686.
3. Tombes, R.M., et al. 1995. G<sub>1</sub> cell cycle arrest apoptosis are induced in NIH 3T3 cells by KN-93, an inhibitor of CaMK-II (the multifunctional Ca<sup>2+</sup>/CaM kinase). *Cell Growth Differ.* 6: 1063-1070.
4. Hama, N., et al. 1995. Calcium/calmodulin-dependent protein kinase II downregulates both calcineurin and protein kinase C-mediated pathways for cytokine gene transcription in human T cells. *J. Exp. Med.* 181: 1217-1222.
5. Tokumitsu, H., et al. 1995. Characterization of a CaM-kinase cascade: molecular cloning and expression of calcium/calmodulin-dependent protein kinase kinase. *J. Biol. Chem.* 270: 19320-19324.
6. Park, I.K., et al. 1995. Activation of Ca<sup>2+</sup>/calmodulin-dependent protein kinase (CaM-kinase) IV by CaM-kinase kinase in Jurkat T lymphocytes. *J. Biol. Chem.* 270: 30464-30469.
7. Anderson, K.A., et al. 1998. Components of a calmodulin-dependent protein kinase cascade. Molecular cloning, functional characterization and cellular localization of Ca<sup>2+</sup>/calmodulin-dependent protein kinase kinase  $\beta$ . *J. Biol. Chem.* 273: 31880-31889.

## CHROMOSOMAL LOCATION

Genetic locus: Camk2 (mouse) mapping to 5 F.

## PRODUCT

CaMKK $\beta$  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  $\mu$ M solution once resuspended using protocol below. Suitable for 50-100 transfections. Also see CaMKK $\beta$  shRNA Plasmid (m): sc-38956-SH and CaMKK $\beta$  shRNA (m) Lentiviral Particles: sc-38956-V as alternate gene silencing products.

For independent verification of CaMKK $\beta$  (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-38956A, sc-38956B and sc-38956C.

## 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  $\mu$ l of the RNase-free water provided. Resuspension of the siRNA duplex in 330  $\mu$ l of RNase-free water makes a 10  $\mu$ M solution in a 10  $\mu$ M Tris-HCl, pH 8.0, 20 mM NaCl, 1 mM EDTA buffered solution.

## APPLICATIONS

CaMKK $\beta$  siRNA (m) is recommended for the inhibition of CaMKK $\beta$  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  $\mu$ M in 66  $\mu$ 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

CaMKK $\beta$  (239CT7.5.3): sc-517319 is recommended as a control antibody for monitoring of CaMKK $\beta$  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).

## RT-PCR REAGENTS

Semi-quantitative RT-PCR may be performed to monitor CaMKK $\beta$  gene expression knockdown using RT-PCR Primer: CaMKK $\beta$  (m)-PR: sc-38956-PR (20  $\mu$ l, 594 bp). Annealing temperature for the primers should be 55-60° C and the extension temperature should be 68-72° C.

## SELECT PRODUCT CITATIONS

1. Balasubramanian, R., et al. 2013. AMP-activated protein kinase as regulator of P2Y<sub>6</sub> receptor-induced Insulin secretion in mouse pancreatic  $\beta$ -cells. *Biochem. Pharmacol.* 85: 991-998.
2. Xi, G., et al. 2019. IGFBP-2 stimulates calcium/calmodulin-dependent protein kinase kinase 2 activation leading to AMP-activated protein kinase induction which is required for osteoblast differentiation. *J. Cell. Physiol.* 234: 23232-23242.

## RESEARCH USE

For research use only, not for use in diagnostic procedures.