

# CaMKK $\alpha$ siRNA (m): sc-29905

## BACKGROUND

The Ca<sup>2+</sup>/calmodulin-dependent protein kinases (CaM kinases) comprise a structurally related subfamily of serine/threonine kinases which include CaMKI, CaMKII and CaMKIV. CaMKII is an ubiquitously expressed serine/threonine protein kinase that is activated by Ca<sup>2+</sup> and calmodulin (CaM) and has been implicated in regulation of the cell cycle and transcription. There are four CaMKII isozymes designated  $\alpha$ ,  $\beta$ ,  $\gamma$  and  $\delta$ , which may or may not be co-expressed in the same tissue type. CaMKIV is stimulated by Ca<sup>2+</sup> and CaM but also requires phosphorylation by a CaMK for full activation. Stimulation of the T cell receptor CD3 signaling complex with an anti-CD3 monoclonal antibody leads to a 10-40 fold increase in CaMKIV activity. An additional kinase, CaMKK, functions to activate CaMKI through the specific phosphorylation of the regulatory Threonine residue at position 177.

## REFERENCES

1. 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.
2. 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.
3. Baltas, L.G., et al. 1995. The cardiac sarcoplasmic reticulum phospholamban kinase is a distinct  $\delta$ -CaM kinase isozyme. *FEBS Lett.* 373: 71-75.
4. 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.

## CHROMOSOMAL LOCATION

Genetic locus: Camkk1 (mouse) mapping to 11 B4.

## PRODUCT

CaMKK $\alpha$  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 $\alpha$  shRNA Plasmid (m): sc-29905-SH and CaMKK $\alpha$  shRNA (m) Lentiviral Particles: sc-29905-V as alternate gene silencing products.

For independent verification of CaMKK $\alpha$  (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-29905A, sc-29905B and sc-29905C.

## 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 $\alpha$  siRNA (m) is recommended for the inhibition of CaMKK $\alpha$  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 $\alpha$  (F-2): sc-17827 is recommended as a control antibody for monitoring of CaMKK $\alpha$  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 $\kappa$  BP-HRP: sc-516102 or m-IgG $\kappa$  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 $\kappa$  BP-FITC: sc-516140 or m-IgG $\kappa$  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 CaMKK $\alpha$  gene expression knockdown using RT-PCR Primer: CaMKK $\alpha$  (m)-PR: sc-29905-PR (20  $\mu$ l, 586 bp). Annealing temperature for the primers should be 55-60° C and the extension temperature should be 68-72° C.

## SELECT PRODUCT CITATIONS

1. Shen, Q.W., et al. 2007. Ca<sup>2+</sup>/calmodulin-dependent protein kinase kinase is involved in AMP-activated protein kinase activation by  $\alpha$ -lipoic acid in C2C12 myotubes. *Am. J. Physiol., Cell Physiol.* 293: C1395-C1403.

## RESEARCH USE

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

## PROTOCOLS

See our web site at [www.scbt.com](http://www.scbt.com) for detailed protocols and support products.