

CaMKI siRNA (r): sc-270482

BACKGROUND

The Ca²⁺/calmodulin-dependent protein kinases (CaM kinases) comprise a structurally related subfamily of serine/threonine kinases which include CaMKI, CaMKII and CaMKIV. CaMKII is a ubiquitously expressed serine/threonine protein kinase that is activated by Ca²⁺ and calmodulin (CaM) and has been implicated in regulation of the cell cycle and transcription. There are four CaMKII isozymes designated α , β , γ and δ , which may or may not be co-expressed in the same tissue type. CaMKIV is stimulated by Ca²⁺ 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. G₁ cell cycle arrest apoptosis are induced in NIH 3T3 cells by KN-93, an inhibitor of CaMK-II (the multifunctional Ca²⁺/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 δ -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.
5. Park, I.K., et al. 1995. Activation of Ca²⁺/calmodulin-dependent protein kinase (CaM-kinase) IV by CaM-kinase kinase in Jurkat T lymphocytes. *J. Biol. Chem.* 270: 30464-30469.
6. Tashima, K., et al. 1996. Overexpression of Ca²⁺/calmodulin-dependent protein kinase II inhibits neurite outgrowth of PC12 cells. *J. Neurochem.* 66: 57-64.

CHROMOSOMAL LOCATION

Genetic locus: Camk1 (rat) mapping to 4q42.

PRODUCT

CaMKI siRNA (r) is a pool of 2 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 CaMKI shRNA Plasmid (r): sc-270482-SH and CaMKI shRNA (r) Lentiviral Particles: sc-270482-V as alternate gene silencing products.

For independent verification of CaMKI (r) gene silencing results, we also provide the individual siRNA duplex components. Each is available as 3.3 nmol of lyophilized siRNA. These include: sc-270482A and sc-270482B.

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

CaMKI siRNA (r) is recommended for the inhibition of CaMKI expression in rat 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

CaMKI (H-8): sc-137225 is recommended as a control antibody for monitoring of CaMKI 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 CaMKI gene expression knockdown using RT-PCR Primer: CaMKI (r)-PR: sc-270482-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.

PROTOCOLS

See our web site at www.scbt.com for detailed protocols and support products.