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CaMKK α siRNA (h2): sc-44293

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

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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: CAMKK1 (human) mapping to 17p13.2.

PRODUCT

CaMKK α siRNA (h2) 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 CaMKK α shRNA Plasmid (h2): sc-44293-SH and CaMKK α shRNA (h2) Lentiviral Particles: sc-44293-V as alternate gene silencing products.

For independent verification of CaMKK α (h2) gene silencing results, we also provide the individual siRNA duplex components. Each is available as 3.3 nmol of lyophilized siRNA. These include: sc-44293A, sc-44293B and sc-44293C.

RESEARCH USE

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

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

CaMKK α siRNA (h2) is recommended for the inhibition of CaMKK α expression in human 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

CaMKK α (6): sc-136280 is recommended as a control antibody for monitoring of 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 α gene expression knockdown using RT-PCR Primer: CaMKK α (h2)-PR: sc-44293-PR (20 μ l). Annealing temperature for the primers should be 55-60° C and the extension temperature should be 68-72° C.

PROTOCOLS

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