SANTA CRUZ BIOTECHNOLOGY, INC.

CaMKIIα siRNA (m): sc-29901

BACKGROUND

The Ca^{2+}/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 Ca^{2+} 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 Ca^{2+} 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_1 cell cycle arrest apoptosis are induced in NIH 3T3 cells by KN-93, an inhibitor of CaMK-II (the multifunctional Ca^{2+}/CaM kinase). Cell Growth Differ. 6: 1063-1070.
2. Baltas, L.G., et al. 1995. The cardiac sarcoplasmic reticulum phospholamban kinase is a distinct δ-CaM kinase isozyme. FEBS Lett. 373: 71-75.
3. 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: Camk2a (mouse) mapping to 18 E1.

PRODUCT

CaMKIIα 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 µM solution once resuspended using protocol below. Suitable for 50-100 transfections. Also see CaMKIIα shRNA Plasmid (m): sc-29901-SH and CaMKIIα shRNA (m) Lentiviral Particles: sc-29901-V as alternate gene silencing products.

For independent verification of CaMKIIα (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-29901A, sc-29901B and sc-29901C.

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 µl of the RNAse-free water provided. Resuspension of the siRNA duplex in 330 µl of RNAse-free water makes a 10 µM solution in a 10 µM Tris-HCl, pH 8.0, 20 mM NaCl, 1 mM EDTA buffered solution.

APPLICATIONS

CaMKIIα siRNA (m) is recommended for the inhibition of CaMKIIα 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 µM in 66 µl. 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

CaMKIIα (A-1): sc-13141 is recommended as a control antibody for monitoring of CaMKIIα 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-IgGκ BP-HRP: sc-516102 or m-IgGκ 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-IgGκ BP-FITC: sc-516140 or m-IgGκ 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 CaMKIIα gene expression knockdown using RT-PCR Primer: CaMKIIα (m)-PR: sc-29901-PR (20 µl, 470 bp). Annealing temperature for the primers should be 55-60° C and the extension temperature should be 68-72° C.

SELECT PRODUCT CITATIONS

1. Bouallegue, A., et al. 2009. CaMKII knockdown attenuates H_2O_2-induced phosphorylation of ERK1/2, PKB/Akt, and IGF-1R in vascular smooth muscle cells. Free Radic. Biol. Med. 47: 858-866.
2. Bouallegue, A., et al. 2013. ET-1-induced growth promoting responses involving ERK1/2 and PKB signaling and Egr-1 expression are mediated by Ca^{2+}/CaM-dependent protein kinase-II in vascular smooth muscle cells. Cell Calcium 54: 428-435.
3. Diniz, L.P., et al. 2014. Astrocyte transforming growth factor β 1 promotes inhibitory synapse formation via CaM kinase II signaling. Glia 62: 1917-1931.
4. Sheng, L., et al. 2015. Neural cell adhesion molecule 2 promotes the formation of filopodia and neurite branching by inducing submembrane increases in Ca^{2+} levels. J. Neurosci. 35: 1739-1752.

RESEARCH USE

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