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CaMKK α shRNA (h2) Lentiviral Particles: sc-44293-V

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 Letts.* 373: 71-75.
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CHROMOSOMAL LOCATION

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

PRODUCT

CaMKK α shRNA (h2) Lentiviral Particles is a pool of concentrated, transduction-ready viral particles containing 3 target-specific constructs that encode 19-25 nt (plus hairpin) shRNA designed to knock down gene expression. Each vial contains 200 μ l frozen stock containing 1.0 x 10⁶ infectious units of virus (IFU) in Dulbecco's Modified Eagle's Medium with 25 mM HEPES pH 7.3. Suitable for 10-20 transductions. Also see CaMKK α siRNA (h2): sc-44293 and CaMKK α shRNA Plasmid (h2): sc-44293-SH as alternate gene silencing products.

STORAGE

Store lentiviral particles at -80° C. Stable for at least one year from the date of shipment. Once thawed, particles can be stored at 4° C for up to one week. Avoid repeated freeze thaw cycles.

APPLICATIONS

CaMKK α shRNA (h2) Lentiviral Particles is recommended for the inhibition of CaMKK α expression in human cells.

SUPPORT REAGENTS

Control shRNA Lentiviral Particles: sc-108080. Available as 200 μ l frozen viral stock containing 1.0 x 10⁶ infectious units of virus (IFU); contains an shRNA construct encoding a scrambled sequence that will not lead to the specific degradation of any known cellular mRNA.

GENE EXPRESSION MONITORING

CaMKK α (R-73): sc-11370 is recommended as a control antibody for monitoring of CaMKK α 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 (secondary) reagents are recommended: 1) Western Blotting: use goat anti-rabbit IgG-HRP: sc-2004 (dilution range: 1:2000-1:100,000) or Cruz Marker™ compatible goat anti-rabbit IgG-HRP: sc-2030 (dilution range: 1:2000-1:5000), Cruz Marker™ Molecular Weight Standards: sc-2035, TBS Blotto A Blocking Reagent: sc-2333 and Western Blotting Luminol Reagent: sc-2048. 2) Immunofluorescence: use goat anti-rabbit IgG-FITC: sc-2012 (dilution range: 1:100-1:400) or goat anti-rabbit IgG-TR: sc-2780 (dilution range: 1:100-1:400) with UltraCruz™ Mounting Medium: sc-24941.

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.

BIOSAFETY

Lentiviral particles can be employed in standard Biosafety Level 2 tissue culture facilities (and should be treated with the same level of caution as with any other potentially infectious reagent). Lentiviral particles are replication-incompetent and are designed to self-inactivate after transduction and integration of shRNA constructs into genomic DNA of target cells.

RESEARCH USE

The purchase of this product conveys to the buyer the nontransferable right to use the purchased amount of the product and all replicates and derivatives for research purposes conducted by the buyer in his laboratory only (whether the buyer is an academic or for-profit entity). The buyer cannot sell or otherwise transfer (a) this product (b) its components or (c) materials made using this product or its components to a third party, or otherwise use this product or its components or materials made using this product or its components for Commercial Purposes.

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