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Cdk1 siRNA (m): sc-142229

BACKGROUND

The activation of signal transduction pathways by growth factors, hormones and neurotransmitters is mediated by the MAP kinases ERK 1 and ERK 2. ERK proteins are regulated by dual phosphorylation at specific tyrosine and threonine sites mapping within a characteristic Thr-Glu-Tyr motif. The protein kinase p56 KKIAMRE is distantly related to the MAP kinase group of proteins and is closely related to p42 KKIALRE. KKIAMRE is predominantly expressed in testis, kidney, brain and lung. KKIAMRE contains the conserved MAP kinase dual phosphorylation motif in the sequence Thr-Asp-Tyr and is activated by treatment of cells by EGF. However, unlike other MAP kinases, the EGF-stimulated kinase activity does not require phosphorylation of KKIAMRE and KKIALRE in the Thr-Asp-Tyr motif.

REFERENCES

1. Boulton, T.G., et al. 1991. Identification of multiple extracellular signal-related kinases (ERKs) with antipeptide antibodies. *Cell Reg.* 2: 357-371.
2. Bouton, T.G., et al. 1991. ERKs: a family of protein-serine/threonine kinases that are activated and tyrosine phosphorylated in response to Insulin and NGF. *Cell* 65: 663-675.
3. Crews, C.M., et al. 1992. Purification of a murine protein-tyrosine/threonine kinase that phosphorylates and activates the Erk-1 gene product: relationship to the fission yeast byr1 gene product. *Proc. Natl. Acad. Sci. USA* 89: 8205-8209.
4. Crews, C.M., et al. 1992. The primary structure of MEK, a protein kinase that phosphorylates the ERK gene product. *Science* 258: 478-480.
5. Meyerson, M., et al. 1992. A family of human cdc2-related protein kinases. *EMBO J.* 11: 2909-2917.
6. Taglienti, C.A., et al. 1996. Molecular cloning of the epidermal growth factor-stimulated protein kinase p56 KKIAMRE. *Oncogene* 13: 2563-2574.

CHROMOSOMAL LOCATION

Genetic locus: Cdk1 (mouse) mapping to 12 C2.

PRODUCT

Cdk1 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 μ M solution once resuspended using protocol below. Suitable for 50-100 transfections. Also see Cdk1 shRNA Plasmid (m): sc-142229-SH and Cdk1 shRNA (m) Lentiviral Particles: sc-142229-V as alternate gene silencing products.

For independent verification of Cdk1 (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-142229A, sc-142229B and sc-142229C.

PROTOCOLS

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

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

Cdk1 siRNA (m) is recommended for the inhibition of Cdk1 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 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

KKIALRE (E-8): sc-515654 is recommended as a control antibody for monitoring of Cdk1 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 Cdk1 gene expression knockdown using RT-PCR Primer: Cdk1 (m)-PR: sc-142229-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.