SANTA CRUZ BIOTECHNOLOGY, INC.

CLK1 siRNA (m): sc-60405

BACKGROUND

The CDC-like kinase 1 (CLK1) dually phosphorylates serine and arginine rich proteins of the spliceosomal complex, which constitutes a network of regulatory mechanisms that enable SR proteins to control RNA splicing. Specifically, CLK1 may mediate the release of specific proteins from nuclear storage sites. Expression of CLK1 may be very low due to a premature stop codon in the mRNA, which leads to nonsense-mediated mRNA decay. CLK1 activity is positively regulated by phosphorylation on either tyrosine residues or serine/threonine residues, and is negatively regulated by steric constraints mediated by the N-terminal domain, and also by phosphorylation on a subset of serine/threonine residues within the catalytic domain.

REFERENCES

1. Duncan, P.I., et al. 1997. *In vivo* regulation of alternative pre-mRNA splicing by the CLK1 protein kinase. Mol. Cell. Biol. 17: 5996-6001.
2. Duncan, P.I., et al. 1998. The CLK2 and CLK3 dual-specificity protein kinases regulate the intranuclear distribution of SR proteins and influence pre-mRNA splicing. Exp. Cell Res. 241: 300-308.
3. Moeslein, F.M., et al. 1999. The CLK family kinases, CLK1 and CLK2, phosphorylate and activate the tyrosine phosphatase, PTP1B. J. Biol. Chem. 274: 26697-26704.
4. Menegay, H.J., et al. 2000. Biochemical characterization and localization of the dual specificity kinase CLK1. J. Cell Sci. 113: 3241-3253.
5. Hartmann, A.M., et al. 2001. Regulation of alternative splicing of human Tau exon 10 by phosphorylation of splicing factors. Mol. Cell. Neurosci. 18: 80-90.
6. Verheyen, G.R., et al. 2004. Microarray analysis of the effect of diesel exhaust particles on *in vitro* cultured macrophages. Toxicol. In Vitro 18: 377-391.
7. Murata, S., et al. 2005. Psychophysiological stress-regulated gene expression in mice. FEBS Lett. 579: 2137-2142.

CHROMOSOMAL LOCATION

Genetic locus: CLK1 (mouse) mapping to 1 C1.3.

PRODUCT

CLK1 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 CLK1 shRNA Plasmid (m): sc-60405-SH and CLK1 shRNA (m) Lentiviral Particles: sc-60405-V as alternate gene silencing products.

For independent verification of CLK1 (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-60405A, sc-60405B and sc-60405C.

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 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

CLK1 siRNA (m) is recommended for the inhibition of CLK1 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

CLK1 (F-12): sc-515897 is recommended as a control antibody for monitoring of CLK1 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 CLK1 gene expression knockdown using RT-PCR Primer: CLK1 (m)-PR: sc-60405-PR (20 µl). 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.