SANTA CRUZ BIOTECHNOLOGY, INC.

CDKL5 siRNA (m): sc-72850

BACKGROUND

Cell cycle progression is controlled in part by a family of cyclin proteins and cyclin-dependent kinases (Cdks). Cdk proteins work in concert with the cyclins to phosphorylate key substrates involved in each phase of cell cycle progression. Another family of proteins, Cdk inhibitors, also plays a role in regulating the cell cycle by binding to cyclin-Cdk complexes and modulating their activity. CDKL5 (cyclin-dependent kinase-like 5) is a 1,030 amino acid protein that belongs to the CMGC Ser/Thr protein kinase family. Expressed in brain, lung, kidney, prostate, ovary, placenta, pancreas and testis, CDKL5 is thought to play a role in cell cycle regulation. Defects in CDKL5 are a cause of several disorders, such as X-linked infantile spasm syndrome and Rett syndrome.

REFERENCES

1. Tao, J., et al. 2004. Mutations in the X-linked cyclin-dependent kinase-like 5 (CDKL5/STK9) gene are associated with severe neurodevelopmental retardation. Am. J. Hum. Genet. 75: 1149-1154.
2. Buoni, S., et al. 2006. Myoclonic encephalopathy in the CDKL5 gene mutation. Clin. Neurophysiol. 117: 223-227.
3. Nectoux, J., et al. 2006. Maternal origin of a novel C-terminal truncation mutation in CDKL5 causing a severe atypical form of Rett syndrome. Clin. Genet. 70: 29-33.
4. Bertani, I., et al. 2006. Functional consequences of mutations in CDKL5, an X-linked gene involved in infantile spasms and mental retardation. J. Biol. Chem. 281: 32048-32056.
5. Archer, H.L., et al. 2006. CDKL5 mutations cause infantile spasms, early onset seizures, and severe mental retardation in female patients. J. Med. Genet. 43: 729-734.
6. Van Esch, H., et al. 2007. Encephalopathy and bilateral cataract in a boy with an interstitial deletion of Xp22 comprising the CDKL5 and NHS genes. Am. J. Med. Genet. A 143A: 364-369.
7. Grosso, S., et al. 2007. Seizures and electroencephalographic findings in CDKL5 mutations: case report and review. Brain Dev. 29: 239-242.
8. Bahi-Buisson, N., et al. 2008. The three stages of epilepsy in patients with CDKL5 mutations. Epilepsia 49: 1027-1037.

CHROMOSOMAL LOCATION

Genetic locus: Cdkl5 (mouse) mapping to X F4.

PRODUCT

CDKL5 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 CDKL5 shRNA Plasmid (m): sc-72850-SH and CDKL5 shRNA (m) Lentiviral Particles: sc-72850-V as alternate gene silencing products.

For independent verification of CDKL5 (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-72850A, sc-72850B and sc-72850C.

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

CDKL5 siRNA (m) is recommended for the inhibition of CDKL5 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

CDKL5 (D-12): sc-376314 is recommended as a control antibody for monitoring of CDKL5 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 CDKL5 gene expression knockdown using RT-PCR Primer: CDKL5 (m)-PR: sc-72850-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.

PROTOCOLS

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