SANTA CRUZ BIOTECHNOLOGY, INC.

CLN5 siRNA (h): sc-60408

SANTA CRUZ BIOTECHNOLOGY

The Power to Question

BACKGROUND

Neuronal ceroid-lipofuscinose (NCL), also designated Batten disease, comprises a group of recessively inherited, progressive neurodegenerative diseases found in children. NCL is characterized by atrophy of the brain and an accumulation of lysosome derived fluorescent bodies found in many cells, especially neurons. Symptoms of NCL include a failure of psychomotor development, seizures, impaired vision and premature death. The eight genes/proteins associated with NCL are designated CLN1-CLN8. Mutations in six of these genes results in a distinct type of NCL-disease; the six genes/proteins are CLN1 (encoding PPT1, a protein thiolesterase), CLN2 (encodeing the serine protease TPP1), CLN3, CLN5, CLN6 and CLN8. A single base duplication mutation in canine and bovine CLN5 has been shown to cause NCL.

REFERENCES

1. Nardocci, N. and Cardona, F. 1998. Neuronal ceroid lipofuscinoses: a review. Ital. J. Neurol. Sci. 19: 271-276.
2. Wisniewski, K.E., et al. 2000. Neuronal ceroid lipofuscinoses: research update. Neurol. Sci. 21: S49-S56.
3. Zhong, N. 2000. Neuronal ceroid lipofuscinoses and possible pathogenic mechanism. Mol. Genet. Metab. 71: 195-206.
4. Heinonen, O., et al. 2000. CLN1 and CLN5, genes for infantile and variant late infantile neuronal ceroid lipofuscinoses, are expressed in the embryonic human brain. J. Comp. Neurol. 426: 406-412.
5. Wisniewski, K.E., et al. 2001. Pheno/genotypic correlations of neuronal ceroid lipofuscinoses. Neurology 57: 576-581.
6. Ranta, S., et al. 2001. Studies of homogenous populations: CLN5 and CLN8. Adv. Genet. 45: 123-140.
7. Melville, S.A., et al. 2005. A mutation in canine CLN5 causes neuronal ceroid lipofuscinosis in Border collie dogs. Genomics 86: 287-294.
8. Mole, S.E., et al. 2005. Correlations between genotype, ultrastructural morphology and clinical phenotype in the neuronal ceroid lipofuscinoses. Neurogenetics 63: 107-126.
9. Pineda-Trujillo, N., et al. 2005. A CLN5 mutation causing an atypical neuronal ceroid lipofuscinosis of juvenile onset. Neurology 64: 740-742.

CHROMOSOMAL LOCATION

Genetic locus: CLN5 (human) mapping to 13q22.3.

PRODUCT

CLN5 siRNA (h) 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 CLN5 shRNA Plasmid (h): sc-60408-SH and CLN5 shRNA (h) Lentiviral Particles: sc-60408-V as alternate gene silencing products.

For independent verification of CLN5 (h) gene silencing results, we also provide the individual siRNA duplex components. Each is available as 3.3 nmol of lyophilized siRNA. These include: sc-60408A, sc-60408B and sc-60408C.

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

CLN5 siRNA (h) is recommended for the inhibition of CLN5 expression in human 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

CLN5 (D-8): sc-374672 is recommended as a control antibody for monitoring of CLN5 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 CLN5 gene expression knockdown using RT-PCR Primer: CLN5 (h)-PR: sc-60408-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.