

CLN2 siRNA (h): sc-45578

BACKGROUND

CLN2, also known as tripeptidyl peptidase I (TPP-I), a member of the family of serine-carboxyl proteinases (S53), plays a crucial role in lysosomal protein degradation and a deficiency in this enzyme leads to fatal neurodegenerative disease. CLN2 is a lysosomal aminopeptidase that sequentially removes tripeptides from small polypeptides and also shows a minor endoprotease activity. In lysosomes, CLN2 proenzyme is converted into a mature enzyme with the assistance of another protease and is able to autoactivate in acidic pH *in vitro* via an unimolecular mechanism.

REFERENCES

1. Golabek, A.A., et al. 2004. Maturation of human tripeptidyl-peptidase I *in vitro*. *J. Biol. Chem.* 279: 31058-31067.
2. Golabek, A.A., et al. 2005. Glycosaminoglycans modulate activation, activity and stability of tripeptidyl-peptidase I *in vitro* and *in vivo*. *J. Biol. Chem.* 280: 7550-7561.
3. Kohan, R., et al. 2005. Palmitoyl protein thioesterase1 (PPT1) and tripeptidyl peptidase-I (TPP-I) are expressed in the human saliva. A reliable and non-invasive source for the diagnosis of infantile (CLN1) and late infantile (CLN2) neuronal ceroid lipofuscinoses. *Clin. Biochem.* 38: 492-494.
4. Oyama, H., et al. 2005. Catalytic residues and substrate specificity of recombinant human tripeptidyl peptidase I (CLN2). *J. Biochem.* 138: 127-134.
5. Sondhi, D., et al. 2005. AAV2-mediated CLN2 gene transfer to rodent and non-human primate brain results in long-term TPP-I expression compatible with therapy for LINCL. *Gene Ther.* 12: 1618-1632.
6. Walus, M., et al. 2005. Ser 475, Glu 272, Asp 276, Asp 327 and Asp 360 are involved in catalytic activity of human tripeptidyl-peptidase I. *FEBS Lett.* 579: 1383-1388.

CHROMOSOMAL LOCATION

Genetic locus: TPP1 (human) mapping to 11p15.4.

PRODUCT

CLN2 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 μ M solution once resuspended using protocol below. Suitable for 50-100 transfections. Also see CLN2 shRNA Plasmid (h): sc-45578-SH and CLN2 shRNA (h) Lentiviral Particles: sc-45578-V as alternate gene silencing products.

For independent verification of CLN2 (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-45578A, sc-45578B and sc-45578C.

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

CLN2 siRNA (h) is recommended for the inhibition of CLN2 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 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

CLN2 (G-3): sc-393961 is recommended as a control antibody for monitoring of CLN2 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 CLN2 gene expression knockdown using RT-PCR Primer: CLN2 (h)-PR: sc-45578-PR (20 μ l, 584 bp). 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.