SANTA CRUZ BIOTECHNOLOGY, INC.

Malin siRNA (h): sc-106193

BACKGROUND

Progressive myoclonic epilepsy type 2 (EPM2), also called Lafora disease, is an autosomal recessive disease characterized by grand mal seizures and/or myoclonus at about 15 years of age. Rapid and severe mental deterioration follows, often with psychotic features. Survival is less than 10 years after onset. Starch-like, endoplasmic reticulum-associated polyglucosans, called Lafora bodies, can be observed in brain, muscle, liver and heart. One cause of Lafora disease is due to mutations in NHLRC1, the gene encoding Malin. Forty-nine different mutations in NHLRC1 have been shown to cause EPM2. Malin, also called NHL repeat-containing protein 1, is a single subunit E3 ubiquitin ligase, containing 6 NHL repeats and 1 RING-type zinc finger. Malin's RING domain is responsible for its ability to mediate ubiquitination. Malin interacts with and polyubiquitinates Laforin, a protein also implicated in EPM2. Malin localizes to the endoplasmic reticulum and, to a lesser extent, in the nucleus. Malin is expressed in brain, cerebellum, spinal cord, medulla, heart, liver, skeletal muscle and pancreas.

REFERENCES

1. Chan, E.M., et al. 2003. Mutations in NHLRC1 cause progressive myoclonus epilepsy. Nat. Genet. 35: 125-127.
2. Chan, E.M., et al. 2004. Progressive myoclonus epilepsy with polyglucosans (Lafora disease): evidence for a third locus. Neurology 63: 565-567.
3. Ianzano, L., et al. 2005. Lafora progressive Myoclonus Epilepsy mutation database-EPM2A and NHLRC1 (EPM2B) genes. Hum. Mutat. 26: 397-397.
4. Lohi, H., et al. 2005. Novel glycogen synthase kinase 3 and ubiquitination pathways in progressive myoclonus epilepsy. Hum. Mol. Genet. 14: 2727-2736.
5. Gentry, M.S., et al. 2005. Insights into Lafora disease: Malin is an E3 ubiquitin ligase that ubiquitinates and promotes the degradation of laforin. Proc. Natl. Acad. Sci. USA 102: 8501-8506.
6. Singh, S., et al. 2006. Novel NHLRC1 mutations and genotype-phenotype correlations in patients with Lafora's progressive myoclonic epilepsy. J. Med. Genet. 43: e48.

CHROMOSOMAL LOCATION

Genetic locus: NHLRC1 (human) mapping to 6p22.3.

PRODUCT

Malin 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 Malin shRNA Plasmid (h): sc-106193-SH and Malin shRNA (h) Lentiviral Particles: sc-106193-V as alternate gene silencing products.

For independent verification of Malin (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-106193A, sc-106193B and sc-106193C.

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

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

Malin (3G6): sc-293401 is recommended as a control antibody for monitoring of Malin 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 Malin gene expression knockdown using RT-PCR Primer: Malin (h)-PR: sc-106193-PR (20 µl). Annealing temperature for the primers should be 55-60° C and the extension temperature should be 68-72° C.

SELECT PRODUCT CITATIONS

1. Sharma, J., et al. 2012. Malin regulates Wnt signaling pathway through degradation of dishevelled2. J. Biol. Chem. 287: 6830-6839.

RESEARCH USE

For research use only, not for use in diagnostic procedures.