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TSEN54 siRNA (m): sc-154715

BACKGROUND

The tRNA-splicing endonuclease complex is responsible for identifying and cleaving pre-tRNA at both 5' and 3' splice sites, thereby releasing introns and free tRNA molecules with 2',3' cyclic phosphates and 5'-OH termini. In addition to its role in pre-tRNA splicing, the heterotetrameric endonuclease complex participates in mRNA processing and, via its association with pre-mRNA processing factors, is thought to link pre-tRNA and pre-mRNA splicing events. TSEN54 (tRNA splicing endonuclease 54 homolog), also known as HsSEN54 (SEN54 homolog) or tRNA-intron endonuclease Sen54, is a 526 amino acid protein belonging to the SEN54 family. Localizing to nucleus, TSEN54 is a member of a complex which identifies and cleaves the splice sites in pre-tRNA, and may also be involved in mRNA processing. Defects in TSEN54 may result in pontocerebellar hypoplasia (PCH) type 4 and 2A, characterized by structural abnormalities to the cerebellum, inferior olive, and ventral pons. TSEN54 exists as two alternatively spliced isoforms.

REFERENCES

1. Paushkin, S.V., et al. 2004. Identification of a human endonuclease complex reveals a link between tRNA splicing and pre-mRNA 3' end formation. *Cell* 117: 311-321.
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3. Budde, B.S., et al. 2008. tRNA splicing endonuclease mutations cause pontocerebellar hypoplasia. *Nat. Genet.* 40: 1113-1118.
4. Cassandrini, D., et al. 2010. Pontocerebellar hypoplasia: clinical, pathologic, and genetic studies. *Neurology* 75: 1459-1464.
5. Namavar, Y., et al. 2011. Clinical, neuroradiological and genetic findings in pontocerebellar hypoplasia. *Brain* 134: 143-156.
6. Namavar, Y., et al. 2011. TSEN54 mutations cause pontocerebellar hypoplasia type 5. *Eur. J. Hum. Genet.* 19: 724-726.
7. Kasher, P.R., et al. 2011. Impairment of the tRNA-splicing endonuclease subunit 54 (*tSEN54*) gene causes neurological abnormalities and larval death in zebrafish models of pontocerebellar hypoplasia. *Hum. Mol. Genet.* 20: 1574-1584.

CHROMOSOMAL LOCATION

Genetic locus: *Tsen54* (mouse) mapping to 11 E2.

PRODUCT

TSEN54 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 μ M solution once resuspended using protocol below. Suitable for 50-100 transfections. Also see TSEN54 shRNA Plasmid (m): sc-154715-SH and TSEN54 shRNA (m) Lentiviral Particles: sc-154715-V as alternate gene silencing products.

For independent verification of TSEN54 (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-154715A, sc-154715B and sc-154715C.

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

TSEN54 siRNA (m) is recommended for the inhibition of TSEN54 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 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

TSEN54 (C-1): sc-374488 is recommended as a control antibody for monitoring of TSEN54 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 TSEN54 gene expression knockdown using RT-PCR Primer: TSEN54 (m)-PR: sc-154715-PR (20 μ l). 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.