SANTA CRUZ BIOTECHNOLOGY, INC.

DPH5 siRNA (m): sc-105315

BACKGROUND

The translation elongation factor 2 in eukaryotes (eEF-2) contains a unique post-translationally modified histidine residue, termed diphthamide, which serves as the only target for diphtheria toxin and *Pseudomonas aeruginosa* exotoxin A. Diphthamide biosynthesis is carried out by five highly conserved proteins, DPH1 to DPH5. The DPH protein family is evolutionarily conserved throughout eukaryotes. The DPH5 gene maps to chromosome one and encodes five isoforms as a result of alternative splicing events. Chromosome 1 is the largest human chromosome spanning about 260 million base pairs. Notable genes located on chromosome 1 include MUTYH, Hutchinson-Gilford progeria, Stickler syndrome, Parkinsons, Gaucher disease and Usher syndrome.

REFERENCES

1. Chen, J.Y., et al. 1988. Biosynthesis of diphthamide in *Saccharomyces cerevisiae*. Partial purification and characterization of a specific S-adenosylmethionine:elongation factor 2 methyltransferase. J. Biol. Chem. 263: 11692-11696.
2. Mattheakis, L.C., et al. 1992. DPH5, a methyltransferase gene required for diphthamide biosynthesis in *Saccharomyces cerevisiae*. Mol. Cell. Biol. 12: 4026-4037.
3. Liu, S., et al. 2004. Identification of the proteins required for biosynthesis of diphthamide, the target of bacterial ADP-ribosylating toxins on translation elongation factor 2. Mol. Cell. Biol. 24: 9487-9497.
4. Weise, A., et al. 2005. New insights into the evolution of chromosome 1. Cytogenet. Genome Res. 108: 217-222.
5. Gregory, S.G., et al. 2006. The DNA sequence and biological annotation of human chromosome 1. Nature 441: 315-321.
6. Liu, S., et al. 2006. Dph3, a small protein required for diphthamide biosynthesis, is essential in mouse development. Mol. Cell. Biol. 26: 3835-3841.
7. Gupta, P.K., et al. 2008. The diphthamide modification on elongation factor-2 renders mammalian cells resistant to ricin. Cell. Microbiol. 10: 1687-1694.
8. Webb, T.R., et al. 2008. Diphthamide modification of eEF2 requires a J-domain protein and is essential for normal development. J. Cell Sci. 121: 3140-3145.

CHROMOSOMAL LOCATION

Genetic locus: Dph5 (mouse) mapping to 3 G1.

PRODUCT

DPH5 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 DPH5 shRNA Plasmid (m): sc-105315-SH and DPH5 shRNA (m) Lentiviral Particles: sc-105315-V as alternate gene silencing products.

For independent verification of DPH5 (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-105315A, sc-105315B and sc-105315C.

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

DPH5 siRNA (m) is recommended for the inhibition of DPH5 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.

RT-PCR REAGENTS

Semi-quantitative RT-PCR may be performed to monitor DPH5 gene expression knockdown using RT-PCR Primer: DPH5 (m)-PR: sc-105315-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.