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SANTA CRUZ BIOTECHNOLOGY, INC.

EYA4 siRNA (m): sc-41953

SANTA CRUZ BIOTECHNOLOGY

The Power to Question

BACKGROUND

A gene of chromosome 6q23 encodes the 640 amino acid protein, EYA4 (eyes absent). EYA is one of four members of the eyes absent family. A 271 amino acid domain at the carboxyl terminal is highly conserved amongst the members of the eyes absent family. EYA4 is expressed in the craniofacial mesenchyme, the dermamyotome, and the limb. The conserved region in other EYA proteins interacts with SIX, DACH, and G-proteins, which regulate transcription in early embryonic development (1,2,3,4). SIX translocates EYA1-3 to the nucleus, and G-proteins can stop this interaction. Premature stop codon mutations in EYA4 cause postlingual, progressive autosomal dominant hearing loss in humans. This shows that EYA4 is also vital to the mature organ of Corti. EYA4 may cause oculo-dento-digital syndrome, based on its expression pattern and map postion.

REFERENCES

1. Borsani, G., et al. 1999. EYA4, a novel vertebrate gene related to *Drosophila* eyes absent. Hum. Mol. Genet. 8: 11-23.
2. Ohto, H., et al. 1999. Cooperation of six and eya in activation of their target genes through nuclear translocation of Eya. Mol. Cell. Biol. 19: 6815-6824.
3. Heanue, T.A., et al. 1999. Synergistic regulation of vertebrate muscle development by Dach2, Eya2, and Six1, homologs of genes required for *Drosophila* eye formation. Genes Dev. 13: 3231-3243.
4. Fan, X., et al. 2000. The alpha subunits of G_z and G_i interact with the eyes absent transcription cofactor Eya2, preventing its interaction with the six class of homeodomain-containing proteins. J. Biol. Chem. 275: 32129-32134.
5. Wayne, S., et al. 2001. Mutations in the transcriptional activator EYA4 cause late-onset deafness at the DFNA10 locus. Hum. Mol. Genet. 10: 195-200.

CHROMOSOMAL LOCATION

Genetic locus: Eya4 (mouse) mapping to 10 A3.

PRODUCT

EYA4 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 EYA4 shRNA Plasmid (m): sc-41953-SH and EYA4 shRNA (m) Lentiviral Particles: sc-41953-V as alternate gene silencing products.

For independent verification of EYA4 (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-41953A, sc-41953B and sc-41953C.

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.

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

EYA4 siRNA (m) is recommended for the inhibition of EYA4 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.

GENE EXPRESSION MONITORING

EYA4 (E-11): sc-393111 is recommended as a control antibody for monitoring of EYA4 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 EYA4 gene expression knockdown using RT-PCR Primer: EYA4 (m)-PR: sc-41953-PR (20 µl). Annealing temperature for the primers should be 55-60° C and the extension temperature should be 68-72° C.