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EYA3 siRNA (h): sc-41950

BACKGROUND

EYA3 (eyes absent homolog 3) is a 573 amino acid protein that localizes to both the nucleus and the cytoplasm and is one of several mammalian homologs of the *Drosophila* Eya (eyes absent) protein. Existing as two alternatively spliced isoforms, EYA3 possesses magnesium-catalyzed phosphatase activity and is thought to play a role in transcriptional regulation during organogenesis. Specifically, EYA3 interacts with proteins such as Six1 and, via this interaction, functions to activate the expression of genes that are involved in cellular pro-liferation and organ development. Upon DNA damage, EYA3 may be phosphorylated by ATM or ATR. The gene encoding EYA3 maps to chromosome 1p35.3, which spans about 260 million base pairs and comprises nearly 8% of the human genome.

REFERENCES

- Zimmerman, J.E., et al. 1997. Cloning and characterization of two vertebrate homologs of the *Drosophila* eyes absent gene. *Genome Res.* 7: 128-141.
- Abdelhak, S., et al. 1997. A human homologue of the *Drosophila* eyes absent gene underlies branchio-oto-renal (BOR) syndrome and identifies a novel gene family. *Nat. Genet.* 15: 157-164.
- Xu, P.X., et al. 1997. Mouse Eya genes are expressed during limb tendon development and encode a transcriptional activation function. *Proc. Natl. Acad. Sci. USA* 94: 11974-11979.
- Borsani, G., et al. 1999. EYA4, a novel vertebrate gene related to *Drosophila* eyes absent. *Hum. Mol. Genet.* 8: 11-23.

CHROMOSOMAL LOCATION

Genetic locus: EYA3 (human) mapping to 1p35.3.

PRODUCT

EYA3 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 EYA3 shRNA Plasmid (h): sc-41950-SH and EYA3 shRNA (h) Lentiviral Particles: sc-41950-V as alternate gene silencing products.

For independent verification of EYA3 (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-41950A, sc-41950B and sc-41950C.

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

EYA3 siRNA (h) is recommended for the inhibition of EYA3 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

EYA3 (G-9): sc-515626 is recommended as a control antibody for monitoring of EYA3 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 EYA3 gene expression knockdown using RT-PCR Primer: EYA3 (h)-PR: sc-41950-PR (20 μ l, 483 bp). Annealing temperature for the primers should be 55-60° C and the extension temperature should be 68-72° C.

SELECT PRODUCT CITATIONS

- Krishnan, N., et al. 2009. Dephosphorylation of the C-terminal tyrosyl residue of the DNA damage-related Histone H2A.X is mediated by the protein phosphatase eyes absent. *J. Biol. Chem.* 284: 16066-16070.

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.