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SANTA CRUZ BIOTECHNOLOGY, INC.

EYA2 siRNA (h): sc-41948

BACKGROUND

A gene on chromosome 20q13.1 encodes EYA2 (eyes absent). EYA2 is one of four members of the eyes absent family. A 271 amino acid domain at the carboxy-terminal is highly conserved amongst the members of the eyes absent family, while the PST (proline-serive-threonin)-rich amino-terminal is highly divergent. EYA2 is expressed relatively late in development in the cytoplasm of extensor tendons and ligaments of the phalangeal elements of the limb, cranial placodes, branchial arches, central nervous system and the developing eye. Pax-3 induces the expression of EYA2 in a cascade that is necessary and sufficient for myogenesis. EYA2, like EYA1, acts as a transcriptional activator in connective tissue patterning through its PST domain, which functions as a transactivation domain. EYA2 is translocated to the nucleus by Six proteins, which interact through their domain and homeodomain with EYA2. EYA2 carboxy-terminal interacts with the $G_{\alpha z}$ and $G_{\alpha i2}$ proteins. This interaction prevents Six proteins from translocating EYA2 to the nucleus.

REFERENCES

1. Xu, P.X., et al. 1997. Mouse EYA homologues of the *Drosophila* eyes absent gene require Pax-6 for expression in lens and nasal placode. Development 124: 219-231.
2. 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.
3. 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.
4. Duncan, M.K., et al. 1997. Eyes absent: a gene family found in several metazoan phyla. Mamm. Genome 8: 479-485. Erratum: Mamm. Genome 8: 877.
5. 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.

CHROMOSOMAL LOCATION

Genetic locus: EYA2 (human) mapping to 20q13.12.

PRODUCT

EYA2 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 EYA2 shRNA Plasmid (h): sc-41948-SH and EYA2 shRNA (h) Lentiviral Particles: sc-41948-V as alternate gene silencing products.

For independent verification of EYA2 (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-41948A, sc-41948B and sc-41948C.

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

EYA2 siRNA (h) is recommended for the inhibition of EYA2 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

EYA2 (F-18): sc-100325 is recommended as a control antibody for monitoring of EYA2 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 EYA2 gene expression knockdown using RT-PCR Primer: EYA2 (h)-PR: sc-41948-PR (20 µl, 419 bp). 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.