

Pax-6 siRNA (r): sc-270113

BACKGROUND

Pax genes contain paired domains with strong homology to genes in *Drosophila* which are involved in programming early development. Lesions in the Pax-6 gene account for most cases of aniridia, a congenital malformation of the eye, chiefly characterized by iris hypoplasia, which can cause blindness. Pax-6 is involved in other anterior segment malformations besides aniridia, such as Peters anomaly, a major error in the embryonic development of the eye with corneal clouding with variable iridolenticulocorneal adhesions. The Pax-6 gene encodes a transcriptional regulator that recognizes target genes through its paired-type DNA-binding domain. The paired domain is composed of two distinct DNA-binding subdomains, the amino-terminal subdomain and the carboxy-terminal subdomain, which bind respective consensus DNA sequences. The human Pax-6 gene produces two alternatively spliced isoforms that have the distinct structure of the paired domain.

REFERENCES

1. Hanson, I.M., et al. 1993. PAX6 mutations in aniridia. *Hum. Mol. Genet.* 2: 915-920.
2. Hanson, I.M., et al. 1994. Mutations at the PAX6 locus are found in heterogeneous anterior segment malformations including Peters anomaly. *Nat. Genet.* 6: 168-173.
3. Azuma, N., et al. 1999. Missense mutation in the alternative splice region of the PAX6 gene in eye anomalies. *Am. J. Hum. Genet.* 65: 656-663.
4. Fic, W., et al. 2007. Eye development under the control of SRp55/B52-mediated alternative splicing of eyeless. *PLoS ONE* 2: e253.

CHROMOSOMAL LOCATION

Genetic locus: Pax6 (rat) mapping to 3q32.

PRODUCT

Pax-6 siRNA (r) 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 Pax-6 shRNA Plasmid (r): sc-270113-SH and Pax-6 shRNA (r) Lentiviral Particles: sc-270113-V as alternate gene silencing products.

For independent verification of Pax-6 (r) gene silencing results, we also provide the individual siRNA duplex components. Each is available as 3.3 nmol of lyophilized siRNA. These include: sc-270113A, sc-270113B and sc-270113C.

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

Pax-6 siRNA (r) is recommended for the inhibition of Pax-6 expression in rat 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

Pax-6 (PAX6): sc-81649 is recommended as a control antibody for monitoring of Pax-6 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 Pax-6 gene expression knockdown using RT-PCR Primer: Pax-6 (r)-PR: sc-270113-PR (20 μ l). Annealing temperature for the primers should be 55-60° C and the extension temperature should be 68-72° C.

SELECT PRODUCT CITATIONS

1. Balakrishnan, S., et al. 2014. Glucose modulates Pax6 expression through the JNK/p38 MAP kinase pathway in pancreatic β -cells. *Life Sci.* 109: 1-7.

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.