SANTA CRUZ BIOTECHNOLOGY, INC.

CRX siRNA (m): sc-38650

BACKGROUND

The cone-rod homeobox-containing gene (CRX) encodes a transcription factor that coordinates the expression of several photoreceptor genes in the developing retina, including opsin and rhodopsin. Specifically, CRX binds the OTX motif (TAATCC/A) upstream from photoreceptor genes. The CRX gene is also expressed in the pinealocytes of the pineal gland and may regulate pineal circadian activity by controlling the expression of melatonin synthesis genes. Furthermore, CRX(-) mice exhibit disruption of circadian rhythms. The human CRX gene maps to chromosome 19q13.3 within the region of the cone-rod dystrophy-2 locus (CORD2). Mutations in the CRX gene are implicated in the visual pathologies of CORD, Leber congenital amaurosis (LCA) and retinitis pigmentosa (RP). All characterized CRX gene mutations produce disease in heterozygotes although there is no known correlation between the pathologic phenotype and genetic mutation. Missense mutations of the CRX gene affect the homeobox domain, whereas frameshift mutations affect the OTX domain.

REFERENCES

1. Furukawa, T., et al. 1997. CRX, a novel OTX-like homeobox gene, shows photoreceptor-specific expression and regulates photoreceptor differentiation. Cell 91: 531-541.
2. Furukawa, T., et al. 1999. Retinopathy and attenuated circadian entrainment in CRX-deficient mice. Nat. Genet. 23: 466-470.
3. Bernard, M., et al. 2001. Transcriptional regulation of the chicken hydroxyindole-O-methyltransferase gene by the cone-rod homeobox-containing protein. J. Neurochem. 79: 248-257.
4. Rivolta, C., et al. 2001. Dominant Leber congenital amaurosis, cone-rod degeneration, and retinitis pigmentosa caused by mutant versions of the transcription factor CRX. Hum. Mutat. 18: 488-498.
5. Rivolta, C., et al. 2001. Novel frameshift mutations in CRX associated with Leber congenital amaurosis. Hum. Mutat. 18: 550-551.
6. Furukawa, A., et al. 2002. The mouse CRX 5'-upstream transgene sequence directs cell-specific and developmentally regulated expression inretinal photoreceptor cells. J. Neurosci. 22: 1640-1647.
7. Gamse, J.T., et al. 2002. OTX5 regulates genes that show circadian expression in the zebrafish pineal complex. Nat. Genet. 30: 117-121.

CHROMOSOMAL LOCATION

Genetic locus: Crx (mouse) mapping to 7 A2.

PRODUCT

CRX 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 CRX shRNA Plasmid (m): sc-38650-SH and CRX shRNA (m) Lentiviral Particles: sc-38650-V as alternate gene silencing products.

For independent verification of CRX (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-38650A, sc-38650B and sc-38650C.

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

CRX siRNA (m) is recommended for the inhibition of CRX 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

CRX (A-9): sc-377138 is recommended as a control antibody for monitoring of CRX 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 CRX gene expression knockdown using RT-PCR Primer: CRX (m)-PR: sc-38650-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.