SANTA CRUZ BIOTECHNOLOGY, INC.

γD-crystallin siRNA (h): sc-40456

BACKGROUND

Crystallins are the major proteins of the vertebrate eye lens, where they maintain the transparency and refractive index of the lens. Crystallins are divided into α, β, and γ families, and the β and γ-crystallins also comprise a superfamily. Crystallins usually contain seven distinctive protein regions, including four homologous motifs, a connecting peptide, and N- and C-terminal extensions. γ-crystallins are structural proteins in the lens, and they exists as monomers which typically lack connecting peptides and terminal extensions. The γ-crystallins include seven closely related proteins, namely γA-, γB-, γC-, γD-, γE-, γF-, and γG-crystallin, as well as the γN and γS-crystallin proteins. The γ-crystallins are differentially regulated after early development, and are involved in cataract formation as a result of either age-related protein degradation or genetic mutation.

REFERENCES

1. Srivastava, O.P., et al. 1998. Degradation of γD- and γs-crystallins in human lenses. Biochem. Biophys. Res. Commun. 253: 288-294.
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3. Klok, E.J., et al. 1998. Regulation of expression within a gene family. The case of the rat γB- and γD-crystallin promoters. J. Biol. Chem. 273: 17206-17215.
4. Stephan, D.A., et al. 1999. Progressive juvenile-onset punctate cataracts caused by mutation of the γD-crystallin gene. Proc. Natl. Acad. Sci. USA 96: 1008-1012.
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6. Pande, A., et al. 2001. Crystal cataracts: human genetic cataract caused by protein crystallization. Proc. Natl. Acad. Sci. USA 98: 6116-6120.
7. Wang, X., et al. 2004. Expression and regulation of α-, β-, and γ-crystallins in mammalian lens epithelial cells. Invest. Ophthalmol. Vis. Sci. 45: 3608-3619.
8. LocusLink Report (LocusID: 1420). http://www.ncbi.nlm.nih.gov/LocusLink

CHROMOSOMAL LOCATION

Genetic locus: CRYGD (human) mapping to 2q33.3.

PRODUCT

γD-crystallin 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 γD-crystallin shRNA Plasmid (h): sc-40456-SH and γD-crystallin shRNA (h) Lentiviral Particles: sc-40456-V as alternate gene silencing products.

For independent verification of γD-crystallin (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-40456A, sc-40456B and sc-40456C.

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

γD-crystallin siRNA (h) is recommended for the inhibition of γD-crystallin 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

γD-crystallin (SB-18): sc-100697 is recommended as a control antibody for monitoring of γD-crystallin 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 γD-crystallin gene expression knockdown using RT-PCR Primer: γD-crystallin (h)-PR: sc-40456-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.