

α A-crystallin siRNA (m): sc-40431

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 compose a superfamily. Crystallins usually contain seven distinct protein regions, including four homologous motifs, a connecting peptide, and N- and C-terminal extensions. α -crystallins consist of three gene products, α A, α B and α C-crystallin, which are members of the small heat shock protein family (HSP 20). They are induced by heat shock, and act as molecular chaperones by holding denatured proteins in large soluble aggregates. However, unlike other molecular chaperones, α -crystallins do not renature these proteins. Expression of α A-crystallin is restricted to the lens. Defects in this gene cause autosomal dominant congenital cataracts (ADCC). The human α B-crystallin gene product is expressed in many tissues, including lens, heart and skeletal muscle. Elevated expression of α B-crystallin is associated with many neurological diseases, and a missense mutation in this gene has co-segregated in a family with a Desmin-related myopathy.

REFERENCES

1. Neuffer, P.D., et al. 1996. Differential expression of B-crystallin and HSP 27 in skeletal muscle during continuous contractile activity. Relationship to myogenic regulatory factors. *J. Biol. Chem.* 271: 24089-24095.
2. Litt, M., et al. 1998. Autosomal dominant congenital cataract associated with a missense mutation in the human α -crystallin gene CRYAA. *Hum. Mol. Genet.* 7: 471-474.
3. Haley, D.A., et al. 1998. The small heat-shock protein, α B-crystallin, has a variable quaternary structure. *J. Mol. Biol.* 277: 27-35.
4. Bova, M.P., et al. 1999. Mutation R120G in α B-crystallin, which is linked to a desmin-related myopathy, results in an irregular structure and defective chaperone-like function. *Proc. Natl. Acad. Sci. USA* 96: 6137-6142.
5. Wang, K., et al. 2000. α -crystallin prevents irreversible protein denaturation and acts cooperatively with other heat-shock proteins to renature the stabilized partially denatured protein in an ATP-dependent manner. *Eur. J. Biochem.* 267: 4705-4712.

CHROMOSOMAL LOCATION

Genetic locus: Cryaa (mouse) mapping to 17 B1.

PRODUCT

α A-crystallin 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 μ M solution once resuspended using protocol below. Suitable for 50-100 transfections. Also see α A-crystallin shRNA Plasmid (m): sc-40431-SH and α A-crystallin shRNA (m) Lentiviral Particles: sc-40431-V as alternate gene silencing products.

For independent verification of α A-crystallin (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-40431A, sc-40431B and sc-40431C.

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

α A-crystallin siRNA (m) is recommended for the inhibition of α A-crystallin expression in mouse 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

α A-crystallin (B-2): sc-28306 is recommended as a control antibody for monitoring of α A-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-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 α A-crystallin gene expression knockdown using RT-PCR Primer: α A-crystallin (m)-PR: sc-40431-PR (20 μ l). 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.