SANTA CRUZ BIOTECHNOLOGY, INC.

αC-crystallin siRNA (m): sc-72423

BACKGROUND

Crystallins are the major proteins expressed in the vertebrate eye lens, where they maintain the transparency and refractive index of the lens. Crystallins are divided into α, β and γ families; β and γ-crystallins compose a superfamily. Crystallins usually contain seven distinctive protein regions, including four homologous motifs, a connecting peptide, and N- and C-terminal extensions. α-crystallins consist of three gene products, αA, αB and αC-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. Research indicates that binding occurs between membranes and αC-crystallin. The binding site appears to be at the polar-apolar interface in membrane protein (MIP26) and αC-crystallin; the lipid bilayer becomes less mobile with αC-crystallin binding.

REFERENCES

1. Neufer, P.D., et al. 1996. Differential expression of β-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, αB-crystallin, has a variable quaternary structure. J. Mol. Biol. 277: 27-35.
4. Bova, M.P., et al. 1999. Mutation R120G in αB-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: Hspb8 (mouse) mapping to 5 F.

PRODUCT

αC-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 µM solution once resuspended using protocol below. Suitable for 50-100 transfections. Also see αC-crystallin shRNA Plasmid (m): sc-72423-SH and αC-crystallin shRNA (m) Lentiviral Particles: sc-72423-V as alternate gene silencing products.

For independent verification of αC-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-72423A, sc-72423B and sc-72423C.

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

αC-crystallin siRNA (m) is recommended for the inhibition of αC-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 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

αC-crystallin (H-5): sc-398395 is recommended as a control antibody for monitoring of αC-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 αC-crystallin gene expression knockdown using RT-PCR Primer: αC-crystallin (m)-PR: sc-72423-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.