

α B-crystallin siRNA (m): sc-40433

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). α -crystallins 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 and defects of this gene cause the development of 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: Cryab (mouse) mapping to 9 A5.3.

PRODUCT

α B-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 B-crystallin shRNA Plasmid (m): sc-40433-SH and α B-crystallin shRNA (m) Lentiviral Particles: sc-40433-V as alternate gene silencing products.

For independent verification of α B-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-40433A, sc-40433B and sc-40433C.

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 B-crystallin siRNA (m) is recommended for the inhibition of α B-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 B-crystallin (F-10): sc-137129 is recommended as a control antibody for monitoring of α B-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 MarkerTM 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 B-crystallin gene expression knockdown using RT-PCR Primer: α B-crystallin (m)-PR: sc-40433-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.