

# $\alpha$ B-crystallin siRNA (h): sc-40432

## 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  $\alpha$ ,  $\beta$  and  $\gamma$  families, and the  $\beta$ - and  $\gamma$ -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.  $\alpha$ -crystallins consist of three gene products,  $\alpha$ A-,  $\alpha$ B- and  $\alpha$ C-crystallin, which are members of the small heat shock protein family (HSP 20).  $\alpha$ -crystallins act as molecular chaperones by holding denatured proteins in large soluble aggregates. However, unlike other molecular chaperones,  $\alpha$ -crystallins do not renature these proteins. Expression of  $\alpha$ A-crystallin is restricted to the lens and defects of this gene cause the development of autosomal dominant congenital cataracts (ADCC). The human  $\alpha$ B-crystallin gene product is expressed in many tissues, including lens, heart and skeletal muscle. Elevated expression of  $\alpha$ 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  $\alpha$ -crystallin gene CRYAA. *Hum. Mol. Genet.* 7: 471-474.
3. Haley, D.A., et al. 1998. The small heat shock protein,  $\alpha$ B-crystallin, has a variable quaternary structure. *J. Mol. Biol.* 277: 27-35.
4. Bova, M.P., et al. 1999. Mutation R120G in  $\alpha$ 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.  $\alpha$ -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 (human) mapping to 11q23.1.

## PRODUCT

$\alpha$ B-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  $\mu$ M solution once resuspended using protocol below. Suitable for 50-100 transfections. Also see  $\alpha$ B-crystallin shRNA Plasmid (h): sc-40432-SH and  $\alpha$ B-crystallin shRNA (h) Lentiviral Particles: sc-40432-V as alternate gene silencing products.

For independent verification of  $\alpha$ B-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-40432A, sc-40432B and sc-40432C.

## 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  $\mu$ l of the RNase-free water provided. Resuspension of the siRNA duplex in 330  $\mu$ l of RNase-free water makes a 10  $\mu$ M solution in a 10  $\mu$ M Tris-HCl, pH 8.0, 20 mM NaCl, 1 mM EDTA buffered solution.

## APPLICATIONS

$\alpha$ B-crystallin siRNA (h) is recommended for the inhibition of  $\alpha$ B-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  $\mu$ M in 66  $\mu$ 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

$\alpha$ B-crystallin (F-10): sc-137129 is recommended as a control antibody for monitoring of  $\alpha$ 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 $\kappa$  BP-HRP: sc-516102 or m-IgG $\kappa$  BP-HRP (Cruz Marker): sc-516102-CM (dilution range: 1:1000-1:10000), Cruz Marker<sup>TM</sup> Molecular Weight Standards: sc-2035, UltraCruz<sup>®</sup> Blocking Reagent: sc-516214 and Western Blotting Luminol Reagent: sc-2048. 2) Immunofluorescence: use m-IgG $\kappa$  BP-FITC: sc-516140 or m-IgG $\kappa$  BP-PE: sc-516141 (dilution range: 1:50-1:200) with UltraCruz<sup>®</sup> Mounting Medium: sc-24941 or UltraCruz<sup>®</sup> Hard-set Mounting Medium: sc-359850.

## RT-PCR REAGENTS

Semi-quantitative RT-PCR may be performed to monitor  $\alpha$ B-crystallin gene expression knockdown using RT-PCR Primer:  $\alpha$ B-crystallin (h)-PR: sc-40432-PR (20  $\mu$ 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](http://www.scbt.com) for detailed protocols and support products.