# γB-crystallin siRNA (m): sc-40453



The Power to Question

### **BACKGROUND**

Crystallins, the major proteins of the vertebrate eye lens, are responsible for maintaining the transparency and the refractive index of the lens. Crystallins are divided into  $\alpha,\,\beta,$  and  $\gamma$  families, all of which usually contain seven distinctive protein regions, including four homologous motifs, one connecting peptide and N- and C-terminal extensions. The  $\gamma$ -crystallin family is comprised of seven closely related proteins designated  $\gamma A$ -,  $\gamma B$ -,  $\gamma C$ -,  $\gamma D$ -,  $\gamma E$ -,  $\gamma F$ - and  $\gamma G$ -crystallin.  $\gamma B$ -crystallin, also known as CRYGB or CRYG2, is a 175 amino acid member of the  $\gamma$ -crystallin family. Functioning as a monomer that has a two-domain  $\beta$  fold,  $\gamma B$ -crystallin, like other members of its family, plays a key role in ensuring the proper structure of the vertebrate eye lens. Defects in the gene encoding  $\gamma B$ -crystallin are associated with the formation of cataracts which are characterized by a clouding of the crystalline lens of the eye.

# **REFERENCES**

- 1. Brakenhoff, R.H., et al. 1990. Human  $\gamma$ -crystallin genes. A gene family on its way to extinction. J. Mol. Biol. 216: 519-532.
- 2. Hearne, C.M., et al. 1991. Trinucleotide repeat polymorphism at the CRYG1 locus. Nucleic Acids Res. 19: 5450.
- 3. Rogaev, E.I., et al. 1996. Linkage of polymorphic congenital cataract to the  $\gamma$ -crystallin gene locus on human chromosome 2q33-35. Hum. Mol. Genet. 5: 699-703.
- 4. Graw, J. 1997. The crystallins: genes, proteins and diseases. Biol. Chem. 378: 1331-1348.
- 5. Stöger, T., et al. 1997. The Cryner element in the murine  $\gamma$ -crystallin promoters interacts with lens proteins. Ophthalmic Res. 29: 161-171.
- 6. Santhiya, S.T., et al. 2002. Novel mutations in the  $\gamma$ -crystallin genes cause autosomal dominant congenital cataracts. J. Med. Genet. 39: 352-358.
- 7. Salim, A., et al. 2003. Homology models of human  $\gamma$ -crystallins: structural study of the extensive charge network in  $\gamma$ -crystallins. Biochem. Biophys. Res. Commun. 300: 624-630.
- Messina-Baas, O.M., et al. 2006. Two affected siblings with nuclear cataract associated with a novel missense mutation in the CRYGD gene. Mol. Vis. 12: 995-1000.

# CHROMOSOMAL LOCATION

Genetic locus: Crygb (mouse) mapping to 1 C2.

### **PRODUCT**

 $\gamma B$ -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  $\mu M$  solution once resuspended using protocol below. Suitable for 50-100 transfections. Also see  $\gamma B$ -crystallin shRNA Plasmid (m): sc-40453-SH and  $\gamma B$ -crystallin shRNA (m) Lentiviral Particles: sc-40453-V as alternate gene silencing products.

For independent verification of  $\gamma B$ -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-40453A, sc-40453B and sc-40453C.

#### STORAGE AND RESUSPENSION

Store lyophilized siRNA duplex at -20 $^{\circ}$  C with desiccant. Stable for at least one year from the date of shipment. Once resuspended, store at -20 $^{\circ}$  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**

 $\gamma B\text{-}crystallin\ siRNA\ (m)\ is\ recommended\ for\ the\ inhibition\ of\ }\gamma B\text{-}crystallin\ expression\ in\ mouse\ cells.}$ 

#### **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  $\mu$ 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-44234, sc-44235, sc-44236, sc-44237 and sc-44238.

### **GENE EXPRESSION MONITORING**

 $\gamma$ B-crystallin (D-5): sc-377056 is recommended as a control antibody for monitoring of  $\gamma$ 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-lgG $\kappa$  BP-HRP: sc-516102 or m-lgG $\kappa$  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-lgG $\kappa$  BP-FITC: sc-516140 or m-lgG $\kappa$  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  $\gamma$ B-crystallin gene expression knockdown using RT-PCR Primer:  $\gamma$ B-crystallin (m)-PR: sc-40453-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 for detailed protocols and support products.

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