

# γD-crystallin siRNA (m): sc-40457

## 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 comprise a superfamily. Crystallins usually contain seven distinctive protein regions, including four homologous motifs, a connecting peptide, and N- and C-terminal extensions.  $\gamma$ -crystallins are structural proteins in the lens, and they exist as monomers which typically lack connecting peptides and terminal extensions. The  $\gamma$ -crystallins include seven closely related proteins, namely  $\gamma$ A-,  $\gamma$ B-,  $\gamma$ C-,  $\gamma$ D-,  $\gamma$ E-,  $\gamma$ F-, and  $\gamma$ G-crystallin, as well as the  $\gamma$ N and  $\gamma$ S-crystallin proteins. The  $\gamma$ -crystallins are differentially regulated after early development, and are involved in cataract formation as a result of either age-related protein degradation or genetic mutation.

## REFERENCES

1. Srivastava, O.P., et al. 1998. Degradation of  $\gamma$ D- and  $\gamma$ S-crystallins in human lenses. *Biochem. Biophys. Res. Commun.* 253: 288-294.
2. Srivastava, O.P., et al. 1998. Purification of  $\gamma$ -crystallin from human lenses by acetone precipitation method. *Curr. Eye Res.* 17: 1074-1081.
3. Klok, E.J., et al. 1998. Regulation of expression within a gene family. The case of the rat  $\gamma$ B- and  $\gamma$ D-crystallin promoters. *J. Biol. Chem.* 273: 17206-17215.
4. Stephan, D.A., et al. 1999. Progressive juvenile-onset punctate cataracts caused by mutation of the  $\gamma$ D-crystallin gene. *Proc. Natl. Acad. Sci. USA* 96: 1008-1012.
5. Jaenicke, R., et al. 2001. Lens crystallins and their microbial homologs: structure, stability, and function. *Crit. Rev. Biochem. Mol. Biol.* 36: 435-499.
6. Pande, A., et al. 2001. Crystal cataracts: human genetic cataract caused by protein crystallization. *Proc. Natl. Acad. Sci. USA* 98: 6116-6120.
7. Wang X, et al. 2004. Expression and regulation of  $\alpha$ -,  $\beta$ -, and  $\gamma$ -crystallins in mammalian lens epithelial cells. *Invest. Ophthalmol. Vis. Sci.* 45: 3608-3619.
8. LocusLink Report (LocusID: 1420). <http://www.ncbi.nlm.nih.gov/LocusLink>

## CHROMOSOMAL LOCATION

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

## PRODUCT

$\gamma$ D-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  $\gamma$ D-crystallin shRNA Plasmid (m): sc-40457-SH and  $\gamma$ D-crystallin shRNA (m) Lentiviral Particles: sc-40457-V as alternate gene silencing products.

For independent verification of  $\gamma$ D-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-40457A, sc-40457B and sc-40457C.

## 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

$\gamma$ D-crystallin siRNA (m) is recommended for the inhibition of  $\gamma$ D-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-44233, sc-44234, sc-44235, sc-44236, sc-44237 and sc-44238.

## GENE EXPRESSION MONITORING

$\gamma$ D-crystallin (SB-18): sc-100697 is recommended as a control antibody for monitoring of  $\gamma$ D-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™ Molecular Weight Standards: sc-2035, UltraCruz® 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® Mounting Medium: sc-24941 or UltraCruz® Hard-set Mounting Medium: sc-359850.

## RT-PCR REAGENTS

Semi-quantitative RT-PCR may be performed to monitor  $\gamma$ D-crystallin gene expression knockdown using RT-PCR Primer:  $\gamma$ D-crystallin (m)-PR: sc-40457-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.