



Phakinin siRNA (m): sc-62795

BACKGROUND

Phakinin, also known as BFSP2 (beaded filament structural protein 2), CP47, CP49 (lens fiber beaded filament protein CP49) or LIFL-L (lens intermediate filament-like light), is a membrane-associated and cytoskeletal intermediate filament (IF) protein specific to the eye lens. IFs are cytoskeletal structures that typically contain a head, rod and tail domain. Unlike most IFs, Phakinin completely lacks the C-terminal tail domain thus contributing to the unique structure of the beaded filament that is specific to the lens. Phakinin is required for the assembly of beaded filaments and cytoskeletal networks that are important for the long-term maintenance of optical properties and transparency of the lens. Phakinin copolymerizes with Filensin, another IF protein, to form the 10-nm filamentous structures of the beaded filaments. Phakinin is also capable of self-assembling into filament-like structures that form thicker bundles. Mutations in the gene encoding Phakinin can result in lens cataract.

REFERENCES

1. Jakobs, P.M., et al. 2000. Autosomal-dominant congenital cataract associated with a deletion mutation in the human beaded filament protein gene BFSP2. *Am. J. Hum. Genet.* 66: 1432-1436.
2. Online Mendelian Inheritance in Man, OMIM™. 2002. Johns Hopkins University, Baltimore, MD. MIM Number: 603212. World Wide Web URL: <http://www.ncbi.nlm.nih.gov/omim/>
3. Sandilands, A., et al. 2003. Knockout of the intermediate filament protein CP49 destabilises the lens fibre cell cytoskeleton and decreases lens optical quality, but does not induce cataract. *Exp. Eye Res.* 76: 385-391.
4. Sandilands, A., et al. 2004. Bfsp2 mutation found in mouse 129 strains causes the loss of CP49' and induces vimentin-dependent changes in the lens fibre cell cytoskeleton. *Exp. Eye Res.* 78: 875-889.
5. Alizadeh, A., et al. 2004. Characterization of a mutation in the lens-specific CP49 in the 129 strain of mouse. *Invest. Ophthalmol. Vis. Sci.* 45: 884-891.
6. Hoehenwarter, W., et al. 2005. Eye lens proteomics: from global approach to detailed information about phakinin and γ E and F crystallin genes. *Proteomics* 5: 245-257.

CHROMOSOMAL LOCATION

Genetic locus: Bfsp2 (mouse) mapping to 9 F1.

PRODUCT

Phakinin 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 Phakinin shRNA Plasmid (m): sc-62795-SH and Phakinin shRNA (m) Lentiviral Particles: sc-62795-V as alternate gene silencing products.

For independent verification of Phakinin (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-62795A, sc-62795B and sc-62795C.

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

Phakinin siRNA (m) is recommended for the inhibition of Phakinin 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

Phakinin (D-7): sc-390848 is recommended as a control antibody for monitoring of Phakinin 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 Marker™ 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 Phakinin gene expression knockdown using RT-PCR Primer: Phakinin (m)-PR: sc-62795-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.