SANTA CRUZ BIOTECHNOLOGY, INC.

AQP0 siRNA (m): sc-42362

BACKGROUND

Aquaporins (AQPs) are a large family of integral membrane channel proteins that facilitate the transport of water through the cell membrane. Aquaporins are widely distributed and are involved in renal water absorption, generation of pulmonary secretions, lacrimation, and the secretion and reabsorption of cerebrospinal fluid and aqueous humor. AQP0 is the most abundant endogenous protein in the plasma membrane of lens fiber cells where it functions not only as a water pore, but it is also involved in fiber-fiber adhesion and is crucial for fiber cell structure and organization. AQP0 contains an additional pore constriction, not seen in any other aquaporin structures, which may be responsible for pore gating. The closed AQP0 pore holds just three water molecules, which are spaced too far apart to form hydrogen bonds with each other. The C-terminal domain of AQP0 undergoes extensive post-translational modification, including many truncations, during lens aging due to the actions of m-calpain, proteases, or non-enzymatic mechanisms. These truncation sites may be involved in the development of cataracts.

REFERENCES

1. Shiels, A., et al. 2000. Disruption of lens fiber cell architecture in mice expressing a chimeric AQP0-LTR protein. FASEB J. 14: 2207-2212.
2. Zampighi, G.A., et al. 2002. Structure of functional single AQP0 channels in phospholipid membranes. J. Mol. Biol. 325: 201-210.
3. Zampighi, G.A., et al. 2002. Micro-domains of AQP0 in lens equatorial fibers. Exp. Eye Res. 75: 505-519.
4. Ball, L.E., et al. 2003. Water permeability of C-termin in the aging human lens. Invest. Ophthalmol. Vis. Sci. 44: 4820-4828.
5. Ball, L.E., et al. 2004. Post-translational modifications of aquaporin 0 (AQP0) lens: spatial and temporal occurrence. Biochemistry 43: 9856-9865.
6. Gonen, T., et al. 2005. Lipid-protein interactions in double-layered two-dimensional AQP0 crystals. Nature 438: 633-638.
7. Hashido, M., et al. 2005. Comparative simulations of aquaporin family: AQP1, AQPZ, AQP0 and GlpF. FEBS Lett. 579: 5549-5552.
8. Han, B.G., et al. 2006. Water transport in AQP0 aquaporin: molecular dynamics studies. J. Mol. Biol. 360: 285-296.

CHROMOSOMAL LOCATION

Genetic locus: Mip (mouse) mapping to 10 D3.

PRODUCT

AQP0 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 µM solution once resuspended using protocol below. Suitable for 50-100 transfections. Also see AQP0 shRNA Plasmid (m): sc-42362-SH and AQP0 shRNA (m) Lentiviral Particles: sc-42362-V as alternate gene silencing products.

For independent verification of AQP0 (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-42362A, sc-42362B and sc-42362C.

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 µl of the RNAse-free water provided. Resuspension of the siRNA duplex in 330 µl of RNAse-free water makes a 10 µM solution in a 10 µM Tris-HCl, pH 8.0, 20 mM NaCl, 1 mM EDTA buffered solution.

APPLICATIONS

AQP0 siRNA (m) is recommended for the inhibition of AQP0 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 µM in 66 µl. 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

AQP0 (B-11): sc-376445 is recommended as a control antibody for monitoring of AQP0 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-IgGκ BP-HRP: sc-516102 or m-IgGκ 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-IgGκ BP-FITC: sc-516140 or m-IgGκ 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 AQP0 gene expression knockdown using RT-PCR Primer: AQP0 (m)-PR: sc-42362-PR (20 µl). 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.