SANTA CRUZ BIOTECHNOLOGY, INC.

AKAP 7 siRNA (h): sc-95270

BACKGROUND

The type II cAMP-protein kinase (PKA) is a multifunctional kinase with a broad range of substrates. Specificity of PKA signaling is thought to be mediated by the compartmentalization of the kinase to specific sites within the cell. To maintain this specific localization, the regulatory (R) subunits (RI and RII) of PKA interact with specific R-anchoring proteins designated AKAPs. AKAP 7 (A-kinase anchor protein 7), also known as AKAP18, is a 104 amino acid protein that belongs to the AKAP family. AKAP 7 is expressed in brain, heart, lung, pancreas and skeletal muscle. AKAP 7 binds PKA to the plasma membrane and permits functional coupling to the L-type calcium channel. Four isoforms exist due to alternative splicing events. It has been suggested that the γ isoform binds RI and may be responsible for positioning PKA via RI and/or RII to regulate PKA-mediated gene transcription in both somatic cells and oocytes.

REFERENCES

1. Trotter, K.W., et al. 1999. Alternative splicing regulates the subcellular localization of A-kinase anchoring protein 18 isoforms. J. Cell Biol. 147: 1481-1492.
2. Scott, J.D., et al. 2000. Coordination of cAMP signaling events through PKA anchoring. Adv. Pharmacol. 47: 175-207.
3. Edwards, A.S. and Scott, J.D. 2000. A-kinase anchoring proteins: protein kinase A and beyond. Curr. Opin. Cell Biol. 12: 217-221.
4. Klussmann, E. and Rosenthal, W. 2001. Role and identification of protein kinase A anchoring proteins in vasopressin-mediated aquaporin-2 translocation. Kidney Int. 60: 446-449.
5. Brown, R.L., et al. 2003. AKAP 7γ is a nuclear RI-binding AKAP. Biochem. Biophys. Res. Commun. 306: 394-401.
6. Henn, V., et al. 2004. Identification of a novel A-kinase anchoring protein 18 isoform and evidence for its role in the vasopressin-induced aquaporin-2 shuttle in renal principal cells. J. Biol. Chem. 279: 26654-26665.

CHROMOSOMAL LOCATION

Genetic locus: AKAP7 (human) mapping to 6q23.2.

PRODUCT

AKAP 7 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 µM solution once resuspended using protocol below. Suitable for 50-100 transfections. Also see AKAP 7 shRNA Plasmid (h): sc-95270-SH and AKAP 7 shRNA (h) Lentiviral Particles: sc-95270-V as alternate gene silencing products.

For independent verification of AKAP 7 (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-95270A, sc-95270B and sc-95270C.

PROTOCOLS

See our web site at www.scbt.com for detailed protocols and support products.

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

AKAP 7 siRNA (h) is recommended for the inhibition of AKAP 7 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 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

AKAP 7 (1F9): sc-517021 is recommended as a control antibody for monitoring of AKAP 7 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 AKAP 7 gene expression knockdown using RT-PCR Primer: AKAP 7 (h)-PR: sc-95270-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.