

AKAP 10 siRNA (h): sc-93998

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 (A-kinase anchoring proteins). AKAP 10 (A kinase anchor protein 10), also known as PRKA10 or D-AKAP2 (dual-specific A kinase-anchoring protein 2), is a 662 amino acid mitochondrial membrane protein that belongs to the AKAP family. AKAP 10 is a dual specificity protein that binds to both type I and type II regulatory subunits of PKA and anchors them to the plasma membrane or the mitochondria. When anchored to the mitochondria, PKA can phosphorylate and, thus, inactivate the proapoptotic protein Bad. This suggests that AKAP 10 indirectly regulates Bad-induced apoptosis by mediating the mitochondrial attachment of PKA. Additionally, AKAP 10 may facilitate G protein-coupled signal transduction and could act as an adaptor in the assembly of multiprotein complexes.

REFERENCES

- Huang, L.J., et al. 1997. D-AKAP2, a novel protein kinase A anchoring protein with a putative RGS domain. *Proc. Natl. Acad. Sci. USA* 94: 11184-11189.
- Wang, L., et al. 2001. Cloning and mitochondrial localization of full-length D-AKAP2, a protein kinase A anchoring protein. *Proc. Natl. Acad. Sci. USA* 98: 3220-3225.
- Perkins, G.A., et al. 2001. PKA, PKC, and AKAP localization in and around the neuromuscular junction. *BMC Neurosci.* 2: 17.
- Hamuro, Y., et al. 2002. Domain organization of D-AKAP2 revealed by enhanced deuterium exchange-mass spectrometry (DXMS). *J. Mol. Biol.* 321: 703-714.
- Gisler, S.M., et al. 2003. PDZK1: II. an anchoring site for the PKA-binding protein D-AKAP2 in renal proximal tubular cells. *Kidney Int.* 64: 1746-1754.

CHROMOSOMAL LOCATION

Genetic locus: AKAP10 (human) mapping to 17p11.2.

PRODUCT

AKAP 10 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 μ M solution once resuspended using protocol below. Suitable for 50-100 transfections. Also see AKAP 10 shRNA Plasmid (h): sc-93998-SH and AKAP 10 shRNA (h) Lentiviral Particles: sc-93998-V as alternate gene silencing products.

For independent verification of AKAP 10 (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-93998A, sc-93998B and sc-93998C.

RESEARCH USE

For research use only, not for use in diagnostic procedures.

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

AKAP 10 siRNA (h) is recommended for the inhibition of AKAP 10 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 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

AKAP 10 (C-8): sc-271074 is recommended as a control antibody for monitoring of AKAP 10 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 AKAP 10 gene expression knockdown using RT-PCR Primer: AKAP 10 (h)-PR: sc-93998-PR (20 μ l, 586 bp). Annealing temperature for the primers should be 55-60° C and the extension temperature should be 68-72° C.

PROTOCOLS

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