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SANTA CRUZ BIOTECHNOLOGY, INC.

AKAP 9 siRNA (h): sc-45364

BACKGROUND

The type II cAMP-dependent protein kinase (PKA) is a multifunctional kinase with a broad range of substrates. Specificity of PKA signaling is mediated by the compartmentalization of the kinase to specific sites within the cell. To maintain this specific localization, the R subunit (RII) of PKA interacts with specific RII-anchoring proteins. This family of proteins is designated A-kinase anchoring proteins (AKAP). AKAP 9, also designated AKAP 450, is a 3,911 amino acid protein which undergoes alternative splicing resulting in multiple isoforms including, AKAP 350 and Yotiao. Research has found AKAP 9 localized to both centrosomes and the Golgi apparatus throughout the cell cycle, and it is suggested that AKAP 9 may function as a scaffolding protein assembling protein kinases and phosphatases based on substrate-specific phosphorylation. An N-terminal sequence from amino acids 1-1626 is identical between the AKAP 9 and Yotiao proteins. The unique C-terminus of the Yotiao isoform contains an additional 12 amino acid sequence not shared with AKAP 9. Yotiao is expressed primarily in pancreas and skeletal muscle. Yotiao interacts with the NR1 sub-unit of the NMDA receptor. Co-assembly of Yotiao/PKAII complexes with NR1 subunits promote cAMP-dependent modulation of NMDA receptor activity at synapses, thereby influencing brain development and synaptic plasticity.

REFERENCES

1. Takahashi, M., et al. 1999. Characterization of a novel giant scaffolding protein, CG-NAP, that anchors multiple signaling enzymes to centrosome and the Golgi apparatus. J. Biol. Chem. 274: 17267-17274.
2. Feliciello, A., et al. 1999. Yotiao protein, a ligand for the NMDA receptor, binds and targets cAMP-dependent protein kinase II(1). FEBS Lett. 464: 174-178.
3. Marx, S.O., et al. 2002. Requirement of a macromolecular signaling complex for β adrenergic receptor modulation of the KCNQ1-KCNE1 potassium channel. Science 295: 496-799.
4. Saucerman, J.J., et al. 2004. Proarrhythmic consequences of a KCNQ1 AKAP-binding domain mutation: computational models of whole cells and heterogeneous tissue. Circ. Res. 95: 1216-1224.

CHROMOSOMAL LOCATION

Genetic locus: AKAP9 (human) mapping to 7q21.2.

PRODUCT

AKAP 9 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 9 shRNA Plasmid (h): sc-45364-SH and AKAP 9 shRNA (h) Lentiviral Particles: sc-45364-V as alternate gene silencing products.

For independent verification of AKAP 9 (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-45364A, sc-45364B and sc-45364C.

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 9 siRNA (h) is recommended for the inhibition of AKAP 9 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 9 (7E12): sc-517030 is recommended as a control antibody for monitoring of AKAP 9 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 9 gene expression knockdown using RT-PCR Primer: AKAP 9 (h)-PR: sc-45364-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.