SANTA CRUZ BIOTECHNOLOGY, INC.

AKAP 79 siRNA (h): sc-29660

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 R subunit (RII) of PKA interacts with specific RII-anchoring proteins. This family of proteins has been designated A-kinase anchoring proteins (AKAP). Members of this family, including MAP2 (microtubule-associated protein 2), neuronally expressed AKAP 79 and AKAP 150, and the DNA binding AKAP 95, display differential tissue specificity and localization. Evidence suggests that AKAP 79 and AKAP 150 are both capable of anchoring PKA to postsynaptic densities (PSD), which are a network of proteins located on the internal surfaces of excitatory synapses.

CHROMOSOMAL LOCATION

Genetic locus: AKAP5 (human) mapping to 14q23.3.

PRODUCT

AKAP 79 siRNA (h) is a pool of 4 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 79 shRNA Plasmid (h): sc-29660-SH and AKAP 79 shRNA (h) Lentiviral Particles: sc-29660-V as alternate gene silencing products.

For independent verification of AKAP 79 (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-29660A, sc-29660B, sc-29660C and sc-29660D.

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 79 siRNA (h) is recommended for the inhibition of AKAP 79 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

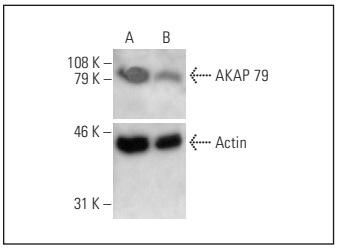
AKAP 79 (D-9): sc-17772 is recommended as a control antibody for monitoring of AKAP 79 gene expression knockdown by Western Blotting (starting dilution 1:200, dilution range 1:100-1:1000) or immunofluorescence (starting dilution 1:50, dilution range 1:50-1:500).

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 79 gene expression knockdown using RT-PCR Primer: AKAP 79 (h)-PR: sc-29660-PR (20 µl, 584 bp). Annealing temperature for the primers should be 55-60° C and the extension temperature should be 68-72° C.

DATA



AKAP 79 siRNA (h): sc-29660. Western blot analysis of AKAP 79 expression in non-transfected control (**A**) and AKAP 79 siRNA transfected (**B**) IMR-32 cells. Blot probed with AKAP 79 (D-9): sc-17772. Actin (I-19): sc-1616 used as specificity and loading control.

SELECT PRODUCT CITATIONS

1. Lynch, M.J., et al. 2005. RNA silencing identifies PDE4D5 as the functionally relevant cAMP phosphodiesterase interacting with β arrestin to control the protein kinase A/AKAP79-mediated switching of the β_2-adrenergic receptor to activation of ERK in HEK293B2 cells. J. Biol. Chem. 280: 33178-33189.
2. Gonzalez de Valdivia, E., et al. 2017. G protein-coupled estrogen receptor 1 (GPER1)/GPR30 increases ERK1/2 activity through PDZ motif-dependent and -independent mechanisms. J. Biol. Chem. 292: 9932-9943.

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.