

AKAP 149 siRNA (h): sc-40301

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. AKAP 149, the human homolog of mouse and rat AKAP 121, is a splice variant of S-AKAP 84 and may be involved in the phosphorylation-dependent regulation of RNA processing.

REFERENCES

1. Scott, J.D., et al. 1990. Type II regulatory subunit dimerization determines the subcellular localization of the cAMP-dependent protein kinase. *J. Biol. Chem.* 265: 21561-21566.
2. Carr, D.W., et al. 1992. Localization of the cAMP-dependent protein kinase to the postsynaptic densities by A-kinase anchoring proteins. Characterization of AKAP 79. *J. Biol. Chem.* 267: 16816-16823.
3. Coghlan, V.M., et al. 1993. A-kinase anchoring proteins: a key to selective activation of cAMP-responsive events? *Mol. Cell. Biochem.* 127: 309-319.
4. Coghlan, V.M., et al. 1994. Cloning and characterization of AKAP 95, a nuclear protein that associates with the regulatory subunit of type II cAMP-dependent protein kinase. *J. Biol. Chem.* 269: 7658-7665.

CHROMOSOMAL LOCATION

Genetic locus: AKAP1 (human) mapping to 17q22.

PRODUCT

AKAP 149 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 149 shRNA Plasmid (h): sc-40301-SH and AKAP 149 shRNA (h) Lentiviral Particles: sc-40301-V as alternate gene silencing products.

For independent verification of AKAP 149 (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-40301A, sc-40301B and sc-40301C.

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 149 siRNA (h) is recommended for the inhibition of AKAP 149 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 149 (B-10): sc-377450 is recommended as a control antibody for monitoring of AKAP 149 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 149 gene expression knockdown using RT-PCR Primer: AKAP 149 (h)-PR: sc-40301-PR (20 μ l, 478 bp). Annealing temperature for the primers should be 55-60° C and the extension temperature should be 68-72° C.

SELECT PRODUCT CITATIONS

1. Chowdhury, S., et al. 2011. Identification of a novel TGF β /PKA signaling transduceome in mediating control of cell survival and metastasis in colon cancer. *PLoS ONE* 6: e19335.
2. Hedrick, E.D., et al. 2013. Differential PKA activation and AKAP association determines cell fate in cancer cells. *J. Mol. Signal.* 8: 10.
3. Pryde, K.R., et al. 2016. PINK1 disables the anti-fission machinery to segregate damaged mitochondria for mitophagy. *J. Cell Biol.* 213: 163-171.

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.