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AKAP 13 siRNA (h): sc-41721

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. The family of RII-anchoring proteins has been designated A-kinase anchoring proteins (AKAP). AKAP 13, also known as BRX (breast cancer nuclear receptor-binding auxiliary protein), LBC (lymphoid blast crisis oncogene), HA-3 or Ht31 (human thyroid-anchoring protein 31), functions as a cAMP-dependent scaffold anchor for PKA and also has Rho-GEF activity. It is known to regulate TLR2 signaling, NF κ B activation, protein kinase D activation and participate in actin stress fiber formation. Seven isoforms exist for AKAP 13 and, depending on the isoform, it localizes to the cytoplasm, nucleus or cell membrane.

REFERENCES

1. Baisamy, L., et al. 2005. Leucine zipper-mediated homo-oligomerization regulates the Rho-GEF activity of AKAP-Lbc. *J. Biol. Chem.* 280: 15405-15412.
2. Lewis, T.E., et al. 2005. Tissue transglutaminase interacts with protein kinase A anchor protein 13 in prostate cancer. *Urol. Oncol.* 23: 407-412.
3. Kino, T., et al. 2006. Rho family Guanine nucleotide exchange factor Brx couples extracellular signals to the glucocorticoid signaling system. *J. Biol. Chem.* 281: 9118-9126.
4. Hearn-Stokes, R., et al. 2006. Expression of the proto-oncoprotein breast cancer nuclear receptor auxiliary factor (Brx) is altered in eutopic endometrium of women with endometriosis. *Fertil. Steril.* 85: 63-70.

CHROMOSOMAL LOCATION

Genetic locus: AKAP13 (human) mapping to 15q25.3.

PRODUCT

AKAP 13 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 13 shRNA Plasmid (h): sc-41721-SH and AKAP 13 shRNA (h) Lentiviral Particles: sc-41721-V as alternate gene silencing products.

For independent verification of AKAP 13 (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-41721A, sc-41721B and sc-41721C.

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 13 siRNA (h) is recommended for the inhibition of AKAP 13 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 13 (A-1): sc-393557 is recommended as a control antibody for monitoring of AKAP 13 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 13 gene expression knockdown using RT-PCR Primer: AKAP 13 (h)-PR: sc-41721-PR (20 μ l, 471 bp). 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.