

AKAP 8L siRNA (h): sc-97872

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 8, also known as AKAP 95, is a nuclear matrix protein predominantly expressed in liver, heart, pancreas, kidney and skeletal muscle. During mitosis, AKAP 8 is recruited to the chromosomes and plays an essential role in mitotic progression. AKAP 8L (AKAP 8-like), also known as HA95 (homologous to AKAP 95 protein), HAP95 (helicase A-binding protein 95), NAKAP or NAKAP95 (neighbor of AKAP 95), is also a nuclear matrix protein and shares 61% homology and 30% identity with AKAP 8. In addition, AKAP 8L forms a complex with AKAP 8 and HDAC3 and is required for the deacetylation of Histone H3 in mitosis.

REFERENCES

1. 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.
2. Collas, P., et al. 1999. The A-kinase-anchoring protein AKAP95 is a multi-valent protein with a key role in chromatin condensation at mitosis. *J. Cell Biol.* 147: 1167-1180.
3. Orstavik, S., et al. 2000. Identification, cloning and characterization of a novel nuclear protein, HA95, homologous to A-kinase anchoring protein 95. *Biol. Cell* 92: 27-37.
4. Westberg, C., et al. 2000. A novel shuttle protein binds to RNA helicase A and activates the retroviral constitutive transport element. *J. Biol. Chem.* 275: 21396-21401.
5. Seki, N., et al. 2000. cDNA cloning of a novel human gene NAKAP95, neighbor of A-kinase anchoring protein 95 (AKAP95) on chromosome 19p13.11-p13.12 region. *J. Hum. Genet.* 45: 31-37.

CHROMOSOMAL LOCATION

Genetic locus: AKAP8L (human) mapping to 19p13.12.

PRODUCT

AKAP 8L 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 8L shRNA Plasmid (h): sc-97872-SH and AKAP 8L shRNA (h) Lentiviral Particles: sc-97872-V as alternate gene silencing products.

For independent verification of AKAP 8L (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-97872A, sc-97872B and sc-97872C.

PROTOCOLS

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

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 8L siRNA (h) is recommended for the inhibition of AKAP 8L 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 8L (A-1): sc-376630 is recommended as a control antibody for monitoring of AKAP 8L 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 (secondary) reagents are recommended: 1) Western Blotting: use goat anti-mouse IgG-HRP: sc-2005 (dilution range: 1:2000-1:32,000) or Cruz Marker™ compatible goat anti-mouse IgG-HRP: sc-2031 (dilution range: 1:2000-1:5000), Cruz Marker™ Molecular Weight Standards: sc-2035, TBS Blotto A Blocking Reagent: sc-2333 and Western Blotting Luminol Reagent: sc-2048. 2) Immunofluorescence: use goat anti-mouse IgG-FITC: sc-2010 (dilution range: 1:100-1:400) or goat anti-mouse IgG-TR: sc-2781 (dilution range: 1:100-1:400) with UltraCruz™ Mounting Medium: sc-24941.

RT-PCR REAGENTS

Semi-quantitative RT-PCR may be performed to monitor AKAP 8L gene expression knockdown using RT-PCR Primer: AKAP 8L (h)-PR: sc-97872-PR (20 μ l). 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.