

AKAP 82 siRNA (m): sc-61963

BACKGROUND

AKAP (A-kinase anchoring proteins) is a family of proteins that interact with the R subunit of PKA to anchor it to specific sites within the cell in order to maintain the specificity of PKA signalling. Members of this family display differential tissue specificity and localization. AKAP 82, also known as PRKA4 (protein kinase A-anchoring protein 4), major sperm fibrous sheath protein or FSC1, is expressed in spermatid during spermiogenesis. AKAP 82 plays an important role in spermatid development, completing the fibrous sheath assembly. AKAP 82 has two binding sites for PKA, one specific for RI α and one that can bind either RI α or RII α . AKAP 82 also binds to AKAP 3. These two proteins together make up most of the insoluble fibrous sheath. In AKAP 82 knockout spermatozoa, a significant reduction or loss of AKAP 3, RII α , SP17 and GAPDS results. Abnormal sperm expression of AKAP 82 may be involved in asthenospermia.

REFERENCES

1. Turner, R.M., et al. 1999. Relationship between sperm motility and the processing and tyrosine phosphorylation of two human sperm fibrous sheath proteins, pro-hAKAP82 and hAKAP82. *Mol. Hum. Reprod.* 5: 816-824.
2. Moss, S.B., et al. 1999. Conservation and function of a bovine sperm A-kinase anchor protein homologous to mouse AKAP 82. *Biol. Reprod.* 61: 335-342.
3. Brown, P.R., et al. 2003. A-kinase anchoring protein 4 binding proteins in the fibrous sheath of the sperm flagellum. *Biol. Reprod.* 68: 2241-2248.
4. Lea, I.A., et al. 2004. Association of sperm protein 17 with A-kinase anchoring protein 3 in flagella. *Reprod. Biol. Endocrinol.* 2: 57.
5. Nipper, R.W., et al. 2005. Differential RNA expression and polyribosome loading of alternative transcripts of the Akap4 gene in murine spermatids. *Mol. Reprod. Dev.* 70: 397-405.
6. Huang, Z., et al. 2005. Changes in intracellular distribution and activity of protein phosphatase PP1 γ 2 and its regulating proteins in spermatozoa lacking AKAP 4. *Biol. Reprod.* 72: 384-392.

CHROMOSOMAL LOCATION

Genetic locus: Akap4 (mouse) mapping to X A1.1.

PRODUCT

AKAP 82 siRNA (m) 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 82 shRNA Plasmid (m): sc-61963-SH and AKAP 82 shRNA (m) Lentiviral Particles: sc-61963-V as alternate gene silencing products.

For independent verification of AKAP 82 (m) gene silencing results, we also provide the individual siRNA duplex components. Each is available as 3.3 nmol of lyophilized siRNA. These include: sc-61963A, sc-61963B and sc-61963C.

RESEARCH USE

For research use only, not for use in diagnostic procedures.

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 82 siRNA (m) is recommended for the inhibition of AKAP 82 expression in mouse 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 82 (25): sc-135827 is recommended as a control antibody for monitoring of AKAP 82 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 82 gene expression knockdown using RT-PCR Primer: AKAP 82 (m)-PR: sc-61963-PR (20 μ l, 498 bp). Annealing temperature for the primers should be 55-60° C and the extension temperature should be 68-72° C.

PROTOCOLS

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