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# AKAP 6 shRNA (m) Lentiviral Particles: sc-45631-V

## 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). AKAP 6 binds to type II regulatory subunits of PKA and targets them to the sarcoplasmic reticulum (SR) and the nuclear membrane. It localizes to the nuclear membrane and SR in heart muscle. AKAP 6 is highly expressed in cardiac muscle, skeletal muscle and to a lesser extent in brain.

## REFERENCES

- 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.
- Coghlan, V.M., et al. 1995. Association of protein kinase A and protein phosphatase 2B with a common anchoring protein. *Science* 267: 108-111.
- Lester, L.B., et al. 1996. Cloning and characterization of a novel A-kinase anchoring protein. AKAP 220, association with testicular peroxisomes. *J. Biol. Chem.* 271: 9460-9465.
- Bers, D.M. 2004. Macromolecular complexes regulating cardiac Ryanodine receptor function. *J. Mol. Cell. Cardiol.* 37: 417-429.
- Carlisle Michel, J.J., et al. 2004. PKA-phosphorylation of PDE4D3 facilitates recruitment of the mAKAP signalling complex. *Biochem. J.* 381: 587-592.
- Pare, G.C., et al. 2005. Nesprin-1 $\alpha$  contributes to the targeting of mAKAP to the cardiac myocyte nuclear envelope. *Exp. Cell Res.* 303: 388-399.

## CHROMOSOMAL LOCATION

Genetic locus: Akap6 (mouse) mapping to 12 C1.

## PRODUCT

AKAP 6 shRNA (m) Lentiviral Particles is a pool of concentrated, transduction-ready viral particles containing 3 target-specific constructs that encode 19-25 nt (plus hairpin) shRNA designed to knock down gene expression. Each vial contains 200  $\mu$ l frozen stock containing  $1.0 \times 10^6$  infectious units of virus (IFU) in Dulbecco's Modified Eagle's Medium with 25 mM HEPES pH 7.3. Suitable for 10-20 transductions. Also see AKAP 6 siRNA (m): sc-45631 and AKAP 6 shRNA Plasmid (m): sc-45631-SH as alternate gene silencing products.

## STORAGE

Store lentiviral particles at  $-80^\circ$  C. Stable for at least one year from the date of shipment. Once thawed, particles can be stored at  $4^\circ$  C for up to one week. Avoid repeated freeze thaw cycles.

## PROTOCOLS

See our web site at [www.scbt.com](http://www.scbt.com) or our catalog for detailed protocols and support products.

## APPLICATIONS

AKAP 6 shRNA (m) Lentiviral Particles is recommended for the inhibition of AKAP 6 expression in mouse cells.

## SUPPORT REAGENTS

Control shRNA Lentiviral Particles: sc-108080. Available as 200  $\mu$ l frozen viral stock containing  $1.0 \times 10^6$  infectious units of virus (IFU); contains an shRNA construct encoding a scrambled sequence that will not lead to the specific degradation of any known cellular mRNA.

## GENE EXPRESSION MONITORING

AKAP 6 (F-16): sc-46057 is recommended as a control antibody for monitoring of AKAP 6 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 donkey anti-goat IgG-HRP: sc-2020 (dilution range: 1:2000-1:100,000) or Cruz Marker<sup>™</sup> compatible donkey anti-goat IgG-HRP: sc-2033 (dilution range: 1:2000-1:5000), Cruz Marker<sup>™</sup> Molecular Weight Standards: sc-2035, TBS Blotto A Blocking Reagent: sc-2333 and Western Blotting Luminol Reagent: sc-2048. 2) Immunofluorescence: use donkey anti-goat IgG-FITC: sc-2024 (dilution range: 1:100-1:400) or donkey anti-goat IgG-TR: sc-2783 (dilution range: 1:100-1:400) with UltraCruz<sup>™</sup> Mounting Medium: sc-24941.

## RT-PCR REAGENTS

Semi-quantitative RT-PCR may be performed to monitor AKAP 6 gene expression knockdown using RT-PCR Primer: AKAP 6 (m)-PR: sc-45631-PR (20  $\mu$ l, 488 bp). Annealing temperature for the primers should be  $55-60^\circ$  C and the extension temperature should be  $68-72^\circ$  C.

## BIOSAFETY

Lentiviral particles can be employed in standard Biosafety Level 2 tissue culture facilities (and should be treated with the same level of caution as with any other potentially infectious reagent). Lentiviral particles are replication-incompetent and are designed to self-inactivate after transduction and integration of shRNA constructs into genomic DNA of target cells.

## RESEARCH USE

The purchase of this product conveys to the buyer the nontransferable right to use the purchased amount of the product and all replicates and derivatives for research purposes conducted by the buyer in his laboratory only (whether the buyer is an academic or for-profit entity). The buyer cannot sell or otherwise transfer (a) this product (b) its components or (c) materials made using this product or its components to a third party, or otherwise use this product or its components or materials made using this product or its components for Commercial Purposes.