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SZABO-SCANDIC HandelsgmbH

Quellenstraße 110, A-1100 Wien

T. +43(0)1 489 3961-0

F. +43(0)1 489 3961-7

mail@szabo-scandic.com

www.szabo-scandic.com

[linkedin.com/company/szaboscandic](https://www.linkedin.com/company/szaboscandic) 

AKAP 9 shRNA (m) Lentiviral Particles: sc-45365-V

BACKGROUND

The type II cAMP-dependent protein kinase (PKA) is a multifunctional kinase with a broad range of substrates. Specificity of PKA signaling is 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 is designated A-kinase anchoring proteins (AKAP). AKAP 9, also designated AKAP 450, is a 3,911 amino acid protein which undergoes alternative splicing resulting in multiple isoforms including, AKAP 350 and Yotiao. Research has found AKAP 9 localized to both centrosomes and the Golgi apparatus throughout the cell cycle, and it is suggested that AKAP 9 may function as a scaffolding protein assembling protein kinases and phosphatases based on substrate-specific phosphorylation. An N-terminal sequence from amino acids 1-1,626 is identical between the AKAP 9 and Yotiao proteins. The unique C-terminus of the Yotiao isoform contains an additional 12 amino acid sequence not shared with AKAP 9. Yotiao is expressed primarily in pancreas and skeletal muscle. Yotiao interacts with the NR1 subunit of the NMDA receptor. Co-assembly of Yotiao/PKAI complexes with NR1 subunits promote cAMP-dependent modulation of NMDA receptor activity at synapses, thereby influencing brain development and synaptic plasticity.

REFERENCES

1. Takahashi, M., et al. 1999. Characterization of a novel giant scaffolding protein, CG-NAP, that anchors multiple signaling enzymes to centrosome and the golgi apparatus. *J. Biol. Chem.* 274: 17267-17274.
2. Feliciello, A., et al. 1999. Yotiao protein, a ligand for the NMDA receptor, binds and targets cAMP-dependent protein kinase II. *FEBS Lett.* 464: 174-178.
3. Marx, S.O., et al. 2002. Requirement of a macromolecular signaling complex for β adrenergic receptor modulation of the KCNQ1-KCNE1 potassium channel. *Science* 295: 496-799.
4. Kanki, H., et al. 2004. A structural requirement for processing the cardiac K^+ channel KCNQ1. *J. Biol. Chem.* 279: 33976-33983.

CHROMOSOMAL LOCATION

Genetic locus: Akap9 (mouse) mapping to 5.

PRODUCT

AKAP 9 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 μ l frozen stock containing 1.0×10^6 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 9 siRNA (m): sc-45365 and AKAP 9 shRNA Plasmid (m): sc-45365-SH as alternate gene silencing products.

STORAGE

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

APPLICATIONS

AKAP 9 shRNA (m) Lentiviral Particles is recommended for the inhibition of AKAP 9 expression in mouse cells.

SUPPORT REAGENTS

Control shRNA Lentiviral Particles: sc-108080. Available as 200 μ l frozen viral stock containing 1.0×10^6 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 9 (L-18): sc-34382 is recommended as a control antibody for monitoring of AKAP 9 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™ compatible donkey anti-goat IgG-HRP: sc-2033 (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 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™ Mounting Medium: sc-24941.

RT-PCR REAGENTS

Semi-quantitative RT-PCR may be performed to monitor AKAP 9 gene expression knockdown using RT-PCR Primer: AKAP 9 (m)-PR: sc-45365-PR (20 μ l, 453 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.

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