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AKAP 12 siRNA (m): sc-44761

BACKGROUND

A-kinase anchor protein 12 (AKAP12), also known as Gravin, Ssecks and AKAP250, is a 1,782 amino acid cell growth related protein that is a member of the AKAP family and contains 3 AKAP domains and binds to the dimeric RII- α regulatory subunit of PKC. AKAP12 is an anchoring protein that mediates the compartmentalization of protein kinase A (PKA) and protein kinase C (PKC) and serves as a scaffold protein in signal transduction. AKAP12 is expressed in endothelial cells, cultured fibroblasts and osteosarcoma cells with localization in the cell cortex and cytoskeleton, but there does not appear to be expression in platelets, leukocytes, monocytic cell lines or peripheral blood cells. Patients with myasthenia gravis (MG) are able to produce antibodies against the C-terminus of AKAP12. The AKAP12 gene is conserved in chimpanzee, Rhesus monkey, canine, bovine, mouse, and rat. The human AKAP12 gene maps to chromosome 6q25.1.

REFERENCES

1. Nauert, J.B., et al. 1997. Gravin, an autoantigen recognized by serum from myasthenia gravis patients, is a kinase scaffold protein. *Curr. Biol.* 7: 52-62.
2. Choi, M.C., et al. 2004. AKAP12/Gravin is inactivated by epigenetic mechanism in human gastric carcinoma and shows growth suppressor activity. *Oncogene* 23: 7095-7103.
3. Streb, J.W., et al. 2004. Multiple promoters direct expression of three AKAP12 isoforms with distinct subcellular and tissue distribution profiles. *J. Biol. Chem.* 279: 56014-56023.

CHROMOSOMAL LOCATION

Genetic locus: Akap12 (mouse) mapping to 10 A1.

PRODUCT

AKAP 12 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 12 shRNA Plasmid (m): sc-44761-SH and AKAP 12 shRNA (m) Lentiviral Particles: sc-44761-V as alternate gene silencing products.

For independent verification of AKAP 12 (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-44761A, sc-44761B and sc-44761C.

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 12 siRNA (m) is recommended for the inhibition of AKAP 12 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 12 (22): sc-293056 is recommended as a control antibody for monitoring of AKAP 12 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 12 gene expression knockdown using RT-PCR Primer: AKAP 12 (m)-PR: sc-44761-PR (20 μ l, 533 bp). Annealing temperature for the primers should be 55-60° C and the extension temperature should be 68-72° C.

SELECT PRODUCT CITATIONS

1. Akakura, S., et al. 2010. Rb-dependent cellular senescence, multinucleation and susceptibility to oncogenic transformation through PKC scaffolding by SSeCKS/AKAP12. *Cell Cycle* 9: 4656-4665.
2. Shin, D.M., et al. 2010. Mycobacterial lipoprotein activates autophagy via TLR2/1/CD14 and a functional vitamin D receptor signalling. *Cell. Microbiol.* 12: 1648-1665.

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.