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# AKAP 12 shRNA (m) Lentiviral Particles: sc-44761-V

## 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- $\alpha$  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.
4. Su, B., et al. 2013. Adhesion-mediated cytoskeletal remodeling is controlled by the direct scaffolding of Src from FAK complexes to lipid rafts by SSeCKS/AKAP12. *Oncogene* 32: 2016-2026.
5. Mostafa, M.R., et al. 2013. Gravin gene expression in acute myeloid leukemia. *Med. Oncol.* 30: 548.

## CHROMOSOMAL LOCATION

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

## PRODUCT

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

## STORAGE

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

## APPLICATIONS

AKAP 12 shRNA (m) Lentiviral Particles is recommended for the inhibition of AKAP 12 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 12 (C-16): sc-48116 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 (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 12 gene expression knockdown using RT-PCR Primer: AKAP 12 (m)-PR: sc-44761-PR (20  $\mu$ l, 533 bp). Annealing temperature for the primers should be  $55-60^\circ\text{C}$  and the extension temperature should be  $68-72^\circ\text{C}$ .

## 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.

## PROTOCOLS

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