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PRAS40 shRNA (h) Lentiviral Particles: sc-44635-V

BACKGROUND

Akt, also known as protein kinase B is one of the major downstream targets of the phosphatidylinositol 3-kinase pathway. This protein kinase has been implicated in Insulin signaling, stimulation of cellular growth, inhibition of apoptosis and transformation of cells. The proline-rich Akt substrate PRAS40, also designated AKT1S1, becomes phosphorylated by activated Akt on Ser or Thr residues in the motif RXRXX(S/T). Phosphorylated PRAS40 subsequently binds 14-3-3 in a sequence-specific manner, thereby inducing such changes as alteration of protein subcellular localization and regulation of intrinsic enzymatic activity. Studies also suggest that PRAS40 phosphorylation and its interaction with pAkt and 14-3-3 may play an important role in neuroprotection mediated by NGF in apoptotic neuronal cell death after cerebral ischemia. PRAS40 maps to human chromosome 19q13.33.

REFERENCES

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5. Saito, A., et al. 2004. Neuroprotective role of a proline-rich Akt substrate in apoptotic neuronal cell death after stroke: relationships with nerve growth factor. *J. Neurosci.* 24: 1584-1593.
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CHROMOSOMAL LOCATION

Genetic locus: AKT1S1 (human) mapping to 19q13.33.

PRODUCT

PRAS40 shRNA (h) 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 PRAS40 siRNA (h): sc-44635 and PRAS40 shRNA Plasmid (h): sc-44635-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

PRAS40 shRNA (h) Lentiviral Particles is recommended for the inhibition of PRAS40 expression in human 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

PRAS40 (H-216): sc-67042 is recommended as a control antibody for monitoring of PRAS40 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 goat anti-rabbit IgG-HRP: sc-2004 (dilution range: 1:2000-1:100,000) or Cruz Marker™ compatible goat anti-rabbit IgG-HRP: sc-2030 (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 goat anti-rabbit IgG-FITC: sc-2012 (dilution range: 1:100-1:400) or goat anti-rabbit IgG-TR: sc-2780 (dilution range: 1:100-1:400) with UltraCruz™ Mounting Medium: sc-24941.

RT-PCR REAGENTS

Semi-quantitative RT-PCR may be performed to monitor PRAS40 gene expression knockdown using RT-PCR Primer: PRAS40 (h)-PR: sc-44635-PR (20 μ l). 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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