SANTA CRUZ BIOTECHNOLOGY, INC.

PRAS40 siRNA (h): sc-44635

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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2. Liu, M.Y., et al. 2002. 14-3-3 interacts with the tumor suppressor tuberin or Akt phosphorylation site(s). Cancer Res. 22: 6475-6480.
3. Chen, H.K., et al. 2003. Interaction of Akt-phosphorylated ataxin-1 with 14-3-3 mediates neurodegeneration in spinocerebellar ataxia type 1. Cell 113: 457-468.
4. Kovacina, K.S., et al. 2003. Identification of a proline-rich Akt substrate as a 14-3-3 binding partner. J. Biol. Chem. 278: 10189-10194.
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.
6. Chan, P.H., 2004. Mitochondria and neuronal death/survival signaling pathways in cerebral ischemia. Neurochem. Res. 29: 1943-1949.
7. Jiang, Y., et al. 2005. Apoptosis and inhibition of the phosphatidylinositol 3-kinase/Akt signaling pathway in the anti-profliferative actions of dehydroepiandrosterone. J. Gastroenterol. 40: 490-497.

CHROMOSOMAL LOCATION

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

PRODUCT

PRAS40 siRNA (h) 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 µM solution once resuspended using protocol below. Suitable for 50-100 transfections. Also see PRAS40 shRNA Plasmid (h): sc-44635-SH and PRAS40 shRNA (h) Lentiviral Particles: sc-44635-V as alternate gene silencing products.

For independent verification of PRAS40 (h) gene silencing results, we also provide the individual siRNA duplex components. Each is available as 3.3 nmol of lyophilized siRNA. These include: sc-44635A, sc-44635B and sc-44635C.

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 µl of the RNAse-free water provided. Resuspension of the siRNA duplex in 330 µl of RNAse-free water makes a 10 µM solution in a 10 µM Tris-HCl, pH 8.0, 20 mM NaCl, 1 mM EDTA buffered solution.

APPLICATIONS

PRAS40 siRNA (h) is recommended for the inhibition of PRAS40 expression in human 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 µM in 66 µl. 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

PRAS40 (199H2R): sc-517639 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 reagents are recommended: 1) Western Blotting: use m-IgGκ BP-HRP: sc-516102 or m-IgGκ 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.

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 µl). Annealing temperature for the primers should be 55-60° C and the extension temperature should be 68-72° C.

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.