SANTA CRUZ BIOTECHNOLOGY, INC.

PIKE siRNA (h): sc-62812

BACKGROUND

PIKE (phosphatidylinositol-3-kinase enhancer), also known as CENTG1 (centaurin-γ-1), AGAP2 or GGAP2, is a 1,192 amino acid protein that is expressed as 2 isoforms, namely PIKE-L, which is brain-specific, and PIKE-A (also known as PIKE-S), which is found throughout the body. Localized to both the nucleus and the cytoplasm, PIKE functions as a GAP (GTPase-activating protein) that is activated by phosphatidylinositol 4,5-bisphosphate (PIP2) and plays an important role in the prevention of neuronal apoptosis. Specifically, PIKE interacts with proteins such as ARF1, ARF5, PLC γ1 and Homer and, via these interactions, regulates endosomal trafficking and protein coupling events. PIKE contains one PH domain, one Miro domain, one Arf-GAP domain and two ANK repeats through which it conveys its protein-binding and GAP activity. While overexpression of PIKE causes tumor growth and invasion, reduced levels of PIKE are associated with neuronal cell death.

REFERENCES

1. Ye, K., et al. 2000. PIKE. A nuclear gtpase that enhances PI3kinase activity and is regulated by protein 4.1N. Cell 103: 919-930.
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3. Ahn, J.Y., et al. 2004. PIKE (phosphatidylinositol 3-kinase enhancer)-A GTPase stimulates Akt activity and mediates cellular invasion. J. Biol. Chem. 279: 16441-16451.
4. Ahn, J.Y., et al. 2004. PIKE-A is amplified in human cancers and prevents apoptosis by up-regulating Akt. Proc. Natl. Acad. Sci. USA 101: 6993-6998.
5. Ahn, J.Y., et al. 2005. PIKE GTPase signaling and function. Int. J. Biol. Sci. 1: 44-50.
6. Liu, X., et al. 2007. PIKE-A is a proto-oncogene promoting cell growth, transformation and invasion. Oncogene 26: 4918-4927.
7. Liu, Z., et al. 2008. Neuroprotective actions of PIKE-L by inhibition of SET proteolytic degradation by asparagine endopeptidase. Mol. Cell 29: 665-678.
8. Liu, R., et al. 2008. Cdk5-mediated regulation of the PIKE-A-Akt pathway and glioblastoma cell invasion. Proc. Natl. Acad. Sci. USA 105: 7570-7575.

CHROMOSOMAL LOCATION

Genetic locus: AGAP2 (human) mapping to 12q14.1.

PRODUCT

PIKE 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 PIKE shRNA Plasmid (h): sc-62812-SH and PIKE shRNA (h) Lentiviral Particles: sc-62812-V as alternate gene silencing products.

For independent verification of PIKE (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-62812A, sc-62812B and sc-62812C.

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

PIKE siRNA (h) is recommended for the inhibition of PIKE 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

PIKE (G-9): sc-166864 is recommended as a control antibody for monitoring of PIKE 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. 2) Immunofluorescence: use m-IgGκ BP-FITC: sc-516140 or m-IgGκ 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 PIKE gene expression knockdown using RT-PCR Primer: PIKE (h)-PR: sc-62812-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.