SANTA CRUZ BIOTECHNOLOGY, INC.

NAPE-PLD siRNA (h): sc-89408

BACKGROUND

NAPE-PLD (N-acyl-phosphatidylethanolamine-hydrolyzing phospholipase D), also known as FMP30, is a 393 amino acid membrane protein and phospholipase D type enzyme that hydrolyzes N-acyl-phosphatidylethanolamines (NAPEs) to produce N-acylethanolamines (NAEs) and phosphatidic acid. Existing as a monomer, NAPE-PLD binds one or two zinc ions per subunit and is stimulated by divalent cations. NAPE-PLD also plays an essential role in the production of anandamide, a protein which acts as a ligand for vanilloid and cannabinoid receptors. The gene encoding NAPE-PLD maps to human chromosome 7, which houses over 1,000 genes and comprises nearly 5% of the human genome. Chromosome 7 has been linked to osteogenesis imperfecta, Pendred syndrome, lissencephaly, citrullinemia and Shwachman-Diamond syndrome.

REFERENCES

1. Tsipouras, P., et al. 1983. Restriction fragment length polymorphism associated with the pro α 2I gene of human type I procollagen. Application to a family with an autosomal dominant form of osteogenesis imperfecta. J. Clin. Invest. 72: 1262-1267.
2. Okamoto, Y., et al. 2004. Molecular characterization of a phospholipase D generating anandamide and its congeners. J. Biol. Chem. 279: 5298-5305.
3. Leung, D., et al. 2006. Inactivation of N-acyl phosphatidylethanolamine phospholipase D reveals multiple mechanisms for the biosynthesis of endocannabinoids. Biochemistry 45: 4720-4726.
4. Reiner, O., et al. 2006. Lissencephaly 1 linking to multiple diseases: mental retardation, neurodegeneration, schizophrenia, male sterility, and more. Neuromolecular Med. 8: 547-565.
5. Gilbert-Dussardier, B. 2006. Williams-Beuren syndrome. Rev. Prat. 56: 2102-2106.
6. Leone, G., et al. 2007. Therapy-related leukemia and myelodysplasia: susceptibility and incidence. Haematologica 92: 1389-1398.
7. Gillum, M.P., et al. 2008. N-acylphosphatidylethanolamine, a gut-derived circulating factor induced by fat ingestion, inhibits food intake. Cell 135: 813-824.

CHROMOSOMAL LOCATION

Genetic locus: NAPEPLD (human) mapping to 7q22.1.

PRODUCT

NAPE-PLD 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 NAPE-PLD shRNA Plasmid (h): sc-89408-SH and NAPE-PLD shRNA (h) Lentiviral Particles: sc-89408-V as alternate gene silencing products.

For independent verification of NAPE-PLD (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-89408A, sc-89408B and sc-89408C.

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

NAPE-PLD siRNA (h) is recommended for the inhibition of NAPE-PLD 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

NAPE-PLD (E-8): sc-514372 is recommended as a control antibody for monitoring of NAPE-PLD 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 NAPE-PLD gene expression knockdown using RT-PCR Primer: NAPE-PLD (h)-PR: sc-89408-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.