

PAFAH1B3 siRNA (h): sc-97212

BACKGROUND

The platelet activating factor (PAF) acetylhydrolases catalyze hydrolysis of the sn-2 ester bond of PAF and related proinflammatory phospholipids and thus attenuate their bioactivity. The family of PAF acetylhydrolases includes one secreted plasma isozyme and two intracellular isozymes. The intracellular isozymes are distinguished by differences in their primary sequence, tissue localization, subunit composition and substrate preferences. The most thoroughly characterized intracellular isoform, PAFAH1B, is a heterotrimeric protein expressed in brain tissue and plays an important role in brain development and function. PAFAH1B is comprised of a regulatory subunit (LIS1) and two homologous (63% identity) catalytic subunits (PAFAH1B2 and PAFAH1B3), which harbor all the activity of the enzyme. The PAFAH1B2 and PAFAH1B3 subunits readily associate with very high affinity to form heterodimers, and this dimerization is essential for both stability and catalytic activity. PAFAH1B3 is also commonly known as PAFAH1B subunit, PAFAH1B subunit γ or PAFAH1B subunit $\alpha 1$.

REFERENCES

1. Moro, F., et al. 1998. The β and γ subunits of the human platelet-activating factor acetyl hydrolase isoform Ib (PAFAH1B2 and PAFAH1B3) map to chromosome 11q23 and 19q13.1, respectively. *Genomics* 51: 157-159.
2. Derewenda, Z.S. and Derewenda, U. 1998. The structure and function of platelet-activating factor acetylhydrolases. *Cell. Mol. Life Sci.* 54: 446-455.
3. Derewenda, Z.S. and Ho, Y.S. 1999. PAF-acetylhydrolases. *Biochim. Biophys. Acta* 1441: 229-236.
4. Sweeney, K.J., et al. 2000. Lissencephaly associated mutations suggest a requirement for the PAFAH1B heterotrimeric complex in brain development. *Mech. Dev.* 92: 263-271.
5. Nothwang, H.G., et al. 2001. Functional hemizygosity of PAFAH1B3 due to a PAFAH1B3-CLK2 fusion gene in a female with mental retardation, ataxia and atrophy of the brain. *Hum. Mol. Genet.* 10: 797-806.
6. Online Mendelian Inheritance in Man, OMIM[™]. 2002. Johns Hopkins University, Baltimore, MD. MIM Number: 603074. World Wide Web URL: <http://www.ncbi.nlm.nih.gov/omim/>

CHROMOSOMAL LOCATION

Genetic locus: PAFAH1B3 (human) mapping to 19q13.2.

PRODUCT

PAFAH1B3 siRNA (h) is a pool of 2 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 μ M solution once resuspended using protocol below. Suitable for 50-100 transfections. Also see PAFAH1B3 shRNA Plasmid (h): sc-97212-SH and PAFAH1B3 shRNA (h) Lentiviral Particles: sc-97212-V as alternate gene silencing products.

For independent verification of PAFAH1B3 (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-97212A and sc-97212B.

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 μ l of the RNase-free water provided. Resuspension of the siRNA duplex in 330 μ l of RNase-free water makes a 10 μ M solution in a 10 μ M Tris-HCl, pH 8.0, 20 mM NaCl, 1 mM EDTA buffered solution.

APPLICATIONS

PAFAH1B3 siRNA (h) is recommended for the inhibition of PAFAH1B3 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 μ M in 66 μ l. 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

PAFAH1B3 (D-8): sc-393612 is recommended as a control antibody for monitoring of PAFAH1B3 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-IgG κ BP-HRP: sc-516102 or m-IgG κ 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-IgG κ BP-FITC: sc-516140 or m-IgG κ 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 PAFAH1B3 gene expression knockdown using RT-PCR Primer: PAFAH1B3 (h)-PR: sc-97212-PR (20 μ l). 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.