SANTA CRUZ BIOTECHNOLOGY, INC.

FAAH siRNA (m): sc-145000

BACKGROUND

FAAH is a membrane-bound enzyme fatty acid amide hydrolase, responsible for the hydrolysis of multiple primary and secondary fatty acid amides, including the neuromodulatory compounds anandamine and oleamide. The degradation of anandamide to arachadonic acid and oleamide to oleic acid, terminates the signaling function of these molecules. FAAH degrades amides and esters with equivalent catalytic efficiency, enabling FAAH to function effectively as both an amidase and esterase. FAAH contributes to anandamide uptake by creating and maintaining an inward concentration gradient for anandamide. A natural single nucleotide polymorphism mutation in human FAAH in its homozygous form is strongly associated with problem drug use. This results in a missense mutation (385C→A) that converts a conserved proline residue to threonine (Pro129→Thr), producing an FAAH variant that displays normal catalytic properties but enhanced sensitivity to proteolytic degradation. Genetic mutations in FAAH consitute an important risk factor for problem drug use. The human FAAH gene maps to chromosome 1p33.

REFERENCES

1. Cravatt, B.F., et al. 1996. Molecular characterization of an enzyme that degrades neuromodulatory fatty-acid amides. Nature 6604: 83-87.
2. Giang, D.K., et al. 1997. Molecular characterization of human and mouse fatty acid amide hydrolases. Proc. Natl. Acad. Sci. USA 6: 2238-2242.
3. Patricelli, M.P., et al. 1999. Fatty acid amide hydrolase competitively degrades bioactive amides and esters through a nonconventialnal catalytic mechanism. Biochemisty 43: 14125-14130.
4. Day, T.A., et al. 2001. Role of fatty acid amide hydrolase in the transport of the endogenous cannabinoid anandamide. Mol. Pharmacol. 6: 1369-1375.
5. Sipe, J.C., et al. 2002. A missense mutation in human fatty acid amide hydrolase associated with problem drug use. Proc. Natl. Acad. Sci. USA 12: 8394-9399.
6. Online Mendelian Inheritance in Man, OMIM™. 2003. Johns Hopkins University, Baltimore, MD. MIM Number: 602935. World Wide Web URL: http://www.ncbi.nlm.nih.gov/omim/

CHROMOSOMAL LOCATION

Genetic locus: Faah (mouse) mapping to 4 D1.

PRODUCT

FAAH siRNA (m) 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 FAAH shRNA Plasmid (m): sc-145000-SH and FAAH shRNA (m) Lentiviral Particles: sc-145000-V as alternate gene silencing products.

For independent verification of FAAH (m) gene silencing results, we also provide the individual siRNA duplex components. Each is available as 3.3 nmol of lyophilized siRNA. These include: sc-145000A, sc-145000B and sc-145000C.

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

FAAH siRNA (m) is recommended for the inhibition of FAAH expression in mouse 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

FAAH (27-Y): sc-100739 is recommended as a control antibody for monitoring of FAAH 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 FAAH gene expression knockdown using RT-PCR Primer: FAAH (m)-PR: sc-145000-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.