SANTA CRUZ BIOTECHNOLOGY, INC.

AFMID siRNA (h): sc-93733

BACKGROUND

AFMID (arylformamidase), also known as probable arylformamidase or kynurenine formamidase (KF), is a 303 amino acid protein belonging to the AFMID family. Encoded by a gene that maps to human chromosome 17q25.3, AFMID catalyzes the hydrolysis of N-formyl-L-kynurenine to L-kynurenine, which is the second step in the conversion of tryptophan to nicotinic acid, NAD(H) and NADP(H). Required for elimination of toxic metabolites, AFMID exists as two alternatively spliced isoforms. AFMID is an atypical example of a mammalian gene whose expression is linked to histone hypoacetylation at its promoter. The Sp1 binding site is essential for KF expression; conversely, the E2F site is superfluous for KF promoter activity. Expression of AFMID and Thymidine Kinase (TK) may be mutually exclusive.

REFERENCES

1. Schuettengruber, B., Doetzlhofer, A., Kroboth, K., Wintersberger, E. and Seiser, C. 2003. Alternate activation of two divergently transcribed mouse genes from a bidirectional promoter is linked to changes in histone modification. J. Biol. Chem. 278: 1784-1793.
2. Dobrovolsky, V.N., Bowyer, J.F., Pabarcus, M.K., Heflich, R.H., Williams, L.D., Doerge, D.R., Arvidsson, B., Bergquist, J. and Casida, J.E. 2005. Effect of arylformamidase (kynurenine formamidase) gene inactivation in mice on enzymatic activity, kynurenine pathway metabolites and phenotype. Biochim. Biophys. Acta 1724: 163-172.
3. Casida, J.E. and Quistad, G.B. 2005. Serine hydrolase targets of organophosphorus toxicants. Chem. Biol. Interact. 157-158: 277-283.
4. Dobrovolsky, V.N., McGarrity, L.J., VonTungeln, L.S., Mittelstaedt, R.A., Morris, S.M., Beland, F.A. and Heflich, R.H. 2005. Micronucleated erythrocyte frequency in control and azidothymidine-treated $Tk^{+/+}$, $Tk^{+/-}$ and $Tk^{-/-}$ mice. Mutat. Res. 570: 227-235.
5. Pabarcus, M.K. and Casida, J.E. 2005. Cloning, expression, and catalytic triad of recombinant arylformamidase. Protein Expr. Purif. 44: 39-44.
6. Bogan, K.L. and Brenner, C. 2008. Nicotinic acid, nicotinamide, and nicotinamide riboside: a molecular evaluation of NAD^+ precursor vitamins in human nutrition. Annu. Rev. Nutr. 28: 115-130.
7. Yoshimura, H., Sakai, T., Kuwahara, Y., Ito, M., Tsuritani, K., Hirasawa, Y. and Nagamatsu, T. 2009. Effects of kynurenine metabolites on mesangial cell proliferation and gene expression. Exp. Mol. Pathol. 87: 70-75.
8. Lin, H., Kwan, A.L. and Dutcher, S.K. 2010. Synthesizing and salvaging NAD: lessons learned from *Chlamydomonas reinhardtii*. PLoS Genet. 6: e1001105
9. Murray, M.F. 2010. Insights into therapy: tryptophan oxidation and HIV infection. Sci. Transl. Med. 2: 32ps23.

CHROMOSOMAL LOCATION

Genetic locus: AFMID (human) mapping to 17q25.3.

PROTOCOLS

See our web site at www.scbt.com for detailed protocols and support products.

PRODUCT

AFMID 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 AFMID shRNA Plasmid (h): sc-93733-SH and AFMID shRNA (h) Lentiviral Particles: sc-93733-V as alternate gene silencing products.

For independent verification of AFMID (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-93733A, sc-93733B and sc-93733C.

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

AFMID siRNA (h) is recommended for the inhibition of AFMID 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.

RT-PCR REAGENTS

Semi-quantitative RT-PCR may be performed to monitor AFMID gene expression knockdown using RT-PCR Primer: AFMID (h)-PR: sc-93733-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.