SANTA CRUZ BIOTECHNOLOGY, INC.

FLAD1 siRNA (m): sc-145197

BACKGROUND

FLAD1 (FAD1 flavin adenine dinucleotide synthetase), also known as FAD1, FADS, PP591 or molybdenum cofactor biosynthesis protein-like, is a 587 amino acid protein where its N-terminus belongs to the moaB/mog family and its C-terminus belongs to the PAPS reductase family. Existing as five alternatively spliced isoforms, FLAD1 localizes to the cytoplasm and utilizes magnesium as a cofactor. FLAD1 is a key enzyme in the metabolic pathway that converts riboflavin into the redox cofactor flavin adenine dinucleotide (FAD). It is suggested that the molybdenum cofactor biosynthesis protein-like region of FLAD1 may not be functional. FLAD1 is encoded by a gene located on human chromosome 1q21.3, which spans 260 million base pairs, contains over 3,000 genes and comprises nearly 8% of the human genome. Aberrations in chromosome 1 are found in a variety of cancers, including head and neck cancer, malignant melanoma and multiple myeloma.

REFERENCES

1. Wu, M., et al. 1995. Cloning and characterization of FAD1, the structural gene for flavin adenine dinucleotide synthetase of *Saccharomyces cerevisiae*. Mol. Cell. Biol. 15: 264-271.
2. Barile, M., et al. 2000. The riboflavin/FAD cycle in rat liver mitochondria. Eur. J. Biochem. 267: 4888-4900.
3. Brizio, C., et al. 2006. Over-expression in *Escherichia coli* and characterization of two recombinant isoforms of human FAD synthetase. Biochem. Biophys. Res. Commun. 344: 1008-1016.
4. Online Mendelian Inheritance in Man, OMIM™. 2006. Johns Hopkins University, Baltimore, MD. MIM Number: 610595. World Wide Web URL: http://www.ncbi.nlm.nih.gov/omim/
5. Chiong, M.A., et al. 2007. Transient multiple acyl-CoA dehydrogenation deficiency in a newborn female caused by maternal riboflavin deficiency. Mol. Genet. Metab. 92: 109-114.
6. Galluccio, M., et al. 2007. Over-expression in *Escherichia coli,* purification and characterization of isoform 2 of human FAD synthetase. Protein Expr. Purif. 52: 175-181.
7. Yruela, I., et al. 2010. Evolutionary divergence of chloroplast FAD synthetase proteins. BMC Evol. Biol. 10: 311.

CHROMOSOMAL LOCATION

Genetic locus: Flad1 (mouse) mapping to 3 F1.

PRODUCT

FLAD1 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 FLAD1 shRNA Plasmid (m): sc-145197-SH and FLAD1 shRNA (m) Lentiviral Particles: sc-145197-V as alternate gene silencing products.

For independent verification of FLAD1 (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-145197A, sc-145197B and sc-145197C.

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

FLAD1 siRNA (m) is recommended for the inhibition of FLAD1 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

FLAD1 (G-4): sc-376819 is recommended as a control antibody for monitoring of FLAD1 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 FLAD1 gene expression knockdown using RT-PCR Primer: FLAD1 (m)-PR: sc-145197-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.