SANTA CRUZ BIOTECHNOLOGY, INC.

FTα siRNA (m): sc-35419

BACKGROUND

Mammalian protein farnesyl transferases are heterodimeric proteins containing two nonidentical α and β subunits that attach farnesyl residues to a cysteine at the fourth position from the COOH terminus of several proteins, including nuclear lamins and p21Ras proteins. The natural substrates contain the Cys-A-A-Xaa recognition sequence, where the A residues are aliphatic and Xaa represents methionine, serine, glutamine or cysteine. The purified farnesyl transferase is an αβ heterodimer. The β subunit binds the peptide substrate while the α subunit is suspected to participate in formation of a stable complex with the substrate farnesyl pyrophosphate. The α subunit is shared with a second prenyltransferase, geranylgeranyl transferase, that attaches 20 carbon geranylgeranyl to Ras-related proteins that terminate in a Cys-A-A-Xaa recognition site in which Xaa is leucine.

REFERENCES

1. Clarke, S., et al. 1988. Posttranslational modification of the Ha-Ras oncogene protein: evidence for a third class of protein carboxyl methyltransferases. Proc. Natl. Acad. Sci. USA 85: 4643-4647.
2. Reiss, Y., et al. 1990. Inhibition of purified p21Ras farnesyl: protein transferase by Cys-AAX tetrapeptides. Cell 62: 81-88.
3. Reiss, Y., et al. 1991. Sequence requirement for peptide recognition by rat brain p21Ras protein farnesyltransferase. Proc. Natl. Acad. Sci. USA 88: 732-736.
4. Chen, W.J., et al. 1991. Cloning and expression of a cDNA encoding the α subunit of rat p21Ras protein farnesyltransferase. Proc. Natl. Acad. Sci. USA 88: 11368-11372.
5. Reiss, Y., et al. 1991. Nonidentical subunits of p21H-Ras farnesyltransferase. J. Biol. Chem. 266: 10672-10677.
4. Moores, S.L., et al. 1991. Sequence dependence of protein isoprenylation. J. Biol. Chem. 266: 14603-14610.
7. Seabra, M.C., et al. 1991. Protein farnesyltransferase and geranylgeranyltransferase share a common α subunit. Cell 65: 429-434.
8. Andres, D.A., et al. 1993. cDNA cloning of the two subunits of human CAAX farnesyltransferase and chromosomal mapping of FNTA and FNTB loci and related sequences. Genomics 18: 105-112.

CHROMOSOMAL LOCATION

Genetic locus: Fnta (mouse) mapping to 8 A2.

PRODUCT

FTα 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 FTα shRNA Plasmid (m): sc-35419-SH and FTα shRNA (m) Lentiviral Particles: sc-35419-V as alternate gene silencing products.

For independent verification of FTα (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-35419A, sc-35419B and sc-35419C.

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

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

FTα (D-5): sc-374262 is recommended as a control antibody for monitoring of FTα 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 FTα gene expression knockdown using RT-PCR Primer: FTα (m)-PR: sc-35419-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.