SANTA CRUZ BIOTECHNOLOGY, INC.

ACOT8 siRNA (h): sc-41058

BACKGROUND

ACOT8 (Acyl-CoA thioesterase 8), also designated TEII p35 or hTE, is a novel human thioesterase known to interact with the HIV protein Nef using yeast two-hybrid screening. Nef is an auxiliary gene of the human immunodeficiency virus (HIV) which facilitates virus replication and enhances infectivity. The roles of Nef in HIV-infected cells are likely mediated by specific interactions with cellular proteins. The interaction between Nef and ACOT8 is correlated with CD4 downregulation, suggesting that ACOT8 may be involved in Nef-mediated CD4 downregulation in HIV-infected cells. ACOT8 is 42% identical to thioesterase II from *Escherichia coli*, and has no significant homology with the two types of animal thioesterases that have previously been cloned (type I and type II thioesterases).

REFERENCES

1. Miller, M.D., et al. 1994. The human immunodeficiency virus-1 Nef gene product: a positive factor for viral infection and replication in primary lymphocytes and macrophages. J. Exp. Med. 179: 101-113.
2. Spina, C.A., et al. 1994. The importance of Nef in the induction of human immunodeficiency virus type 1 replication from primary quiescent CD4 lymphocytes. J. Exp. Med. 179: 115-123.
3. Smith, S. 1994. The animal fatty acid synthase: one gene, one polypeptide, seven enzymes. FASEB J. 8: 1248-1259.
4. Schwartz, O., et al. 1995. Human immunodeficiency virus type 1 Nef increases the efficiency of reverse transcription in the infected cell. J. Virol. 69: 4053-4059.
5. Aiken, C., et al. 1995. Nef stimulates human immunodeficiency virus type 1 proviral DNA synthesis. J. Virol. 69: 5048-5056.
6. Liu, L.X., et al. 1997. Binding of HIV-1 Nef to a novel thioesterase enzyme correlates with Nef-mediated CD4 down-regulation. J. Biol. Chem. 272: 13779-13785.
7. SWISS-PROT/TrEMBL (O14734). World Wide Web URL: http://www.uniprot.org/uniprot/O14734

CHROMOSOMAL LOCATION

Genetic locus: ACOT8 (human) mapping to 20q13.12.

PRODUCT

ACOT8 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 ACOT8 shRNA Plasmid (h): sc-41058-SH and ACOT8 shRNA (h) Lentiviral Particles: sc-41058-V as alternate gene silencing products.

For independent verification of ACOT8 (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-41058A, sc-41058B and sc-41058C.

RESEARCH USE

For research use only, not for use in diagnostic procedures.

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

ACOT8 siRNA (h) is recommended for the inhibition of ACOT8 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.

GENE EXPRESSION MONITORING

ACOT8 (C-3): sc-7343 is recommended as a control antibody for monitoring of ACOT8 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 ACOT8 gene expression knockdown using RT-PCR Primer: ACOT8 (h)-PR: sc-41058-PR (20 µl). Annealing temperature for the primers should be 55-60° C and the extension temperature should be 68-72° C.

PROTOCOLS

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