SANTA CRUZ BIOTECHNOLOGY, INC.

CD3EAP siRNA (h): sc-72829

BACKGROUND

RNA polymerase I (Pol I) is a multi-subunit complex responsible for catalyzing the transcription of DNA into RNA, specifically via the synthesis of ribosomal RNA precursors. CD3EAP, also known as ASE1, CAST or PAF49, is a 510 amino acid protein that localizes to the nucleus and belongs to the eukaryotic RPA34 RNA polymerase subunit family. Existing as two alternatively spliced isoforms, CD3EAP functions as a component of the Pol I complex, specifically exhibiting DNA-dependent RNA polymerase activity and effectively catalyzing the the conversion of a nucleoside triphosphate into a diphosphate, thereby transcribing DNA into RNA. Isoform 2 of CD3EAP may be a component of the T cell receptor (TCR) complex and is subject to tyrosine phosphorylation in response to TCR stimulation. Both isoforms of CD3EAP are subject to DNA damage-dependent phosphorylation, probably by Atm or ATR.

REFERENCES

1. Whitehead, C.M., et al. 1997. ASE-1: a novel protein of the fibrillar centres of the nucleolus and nucleolus organizer region of mitotic chromosomes. Chromosoma 106: 493-502.
2. Yamazaki, T., et al. 1999. CAST, a novel CD3ε-binding protein transducing activation signal for interleukin-2 production in T cells. J. Biol. Chem. 274: 18173-18180.
3. Yamamoto, K., et al. 2004. Multiple protein-protein interactions by RNA polymerase I-associated factor PAF49 and role of PAF49 in rRNA transcription. Mol. Cell. Biol. 24: 6338-6349.
4. Online Mendelian Inheritance in Man, OMIM™. 2004. Johns Hopkins University, Baltimore, MD. MIM Number: 107325. World Wide Web URL: http://www.ncbi.nlm.nih.gov/omim/
5. Vogel, U., et al. 2005. Effect of polymorphisms in XPD, RAI, ASE-1 and ERCC1 on the risk of basal cell carcinoma among Caucasians after age 50. Cancer Detect. Prev. 29: 209-214.
6. Panov, K.I., et al. 2006. RNA polymerase I-specific subunit CAST/hPAF49 has a role in the activation of transcription by upstream binding factor. Mol. Cell. Biol. 26: 5436-5448.

CHROMOSOMAL LOCATION

Genetic locus: CD3EAP (human) mapping to 19q13.32.

PRODUCT

CD3EAP 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 CD3EAP shRNA Plasmid (h): sc-72829-SH and CD3EAP shRNA (h) Lentiviral Particles: sc-72829-V as alternate gene silencing products.

For independent verification of CD3EAP (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-72829A, sc-72829B and sc-72829C.

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

CD3EAP siRNA (h) is recommended for the inhibition of CD3EAP 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

CD3EAP (A-9): sc-393818 is recommended as a control antibody for monitoring of CD3EAP 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 CD3EAP gene expression knockdown using RT-PCR Primer: CD3EAP (h)-PR: sc-72829-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.