SANTA CRUZ BIOTECHNOLOGY, INC.

CREST siRNA (h): sc-60441

BACKGROUND

The calcium-responsive transactivator (CREST, SS18L1) protein localizes to nuclear bodies and is required for the normal development of neuronal dendritic trees. CREST contains a multifunctional domain (MFD), which mediates transcription transactivation, nuclear body targeting, and dimerization. CREST interacts with adenosine 3', 5'-monophosphate (cAMP) response element-binding protein (CREB)-binding protein (CBP) to regulate neuronal morphogenesis. CREST exhibits ubiquitous expression, with lowest levels observed in the spleen. Mice with a targeted disruption of the SS18L1 gene (CREST gene) are viable despite defects in cortical and hippocampal dendrite development. Cortical neurons from Crest-mutant mice are compromised in calcium-dependent dendritic growth, which leads to the conclusion that calcium activation of CREST-mediated transcription helps regulate neuronal morphogenesis.

REFERENCES

1. Ishikawa, K., et al. 1998. Prediction of the coding sequences of unidentified human genes. X. The complete sequences of 100 new cDNA clones from brain which can code for large proteins *in vitro*. DNA Res. 5: 169-176.
2. de Bruijn, D.R., et al. 2001. Mapping and characterization of the mouse and human SS18 genes, two human SS18-like genes and a mouse SS18 pseudogene. Cytogenet. Cell Genet. 92: 310-319.
3. Storlazzi, C.T., et al. 2003. A novel fusion gene, SS18L1/SSX1, in synovial sarcoma. Genes Chromosomes Cancer 37: 195-200.
4. Aizawa, H., et al. 2004. Dendrite development regulated by CREST, a calcium-regulated transcriptional activator. Science 303: 197-202.
5. Pradhan, A., et al. 2004. The calcium-responsive transactivator recruits CREB binding protein to nuclear bodies. Neurosci. Lett. 370: 191-195.
6. Pradhan, A., et al. 2005. A multifunctional domain of the calcium-responsive transactivator (CREST) that inhibits dendritic growth in cultured neurons. J. Biol. Chem. 280: 24738-24743.
7. de Bruijn, D.R., et al. 2006. Common origin of the human synovial sarcoma associated SS18 and SS18L1 gene loci. Cytogenet. Genome Res. 112: 222-226.

CHROMOSOMAL LOCATION

Genetic locus: SS18L1 (human) mapping to 20q13.33.

PRODUCT

CREST 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 CREST shRNA Plasmid (h): sc-60441-SH and CREST shRNA (h) Lentiviral Particles: sc-60441-V as alternate gene silencing products.

For independent verification of CREST (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-60441A, sc-60441B and sc-60441C.

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

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

CREST (D-7): sc-515827 is recommended as a control antibody for monitoring of CREST 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 CREST gene expression knockdown using RT-PCR Primer: CREST (h)-PR: sc-60441-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.