SANTA CRUZ BIOTECHNOLOGY, INC.

CSA siRNA (m): sc-37793

BACKGROUND

Nucleotide excision repair of DNA lesions occurs more rapidly and at a higher frequency on the template, or the transcribed, strand of DNA and to a much lesser extent on the coding, or the non-transcribed, strand or on transcriptionally inactive DNA. CSA and CSB are two related genes that are responsible for directing this preferential DNA repair pattern, known as transcriptional-repair coupling. Cells from patients with the UV-sensitive nucleotide excision repair disorder Cockayne's syndrome (CS) have specific mutations affecting these genes and results in defects of the preferential repair on the transcribed strand of activated genes. CSA is a protein that belongs in the "WD-repeat" family of proteins. CSB, which is also designated excision repair cross-complementing protein-6 (ERCC-6), is the homolog of the yeast Rad26 protein. CSB belongs in the SWI/SNF family of proteins as it contains helicase motifs and ATPase activity.

REFERENCES

1. Troelstra, C., et al. 1992. ERCC6, a member of a subfamily of putative helicases, is involved in Cockayne's syndrome and preferential repair of active genes. Cell 71: 939-953.
2. Troelstra, C., et al. 1993. Structure and expression of the excision repair gene ERCC6, involved in the human disorder Cockayne's syndrome group B. Nucleic Acids Res. 21: 419-426.
3. Henning, K.A., et al. 1995. The Cockayne syndrome group A gene encodes a WD repeat protein that interacts with CSB protein and a subunit of RNA polymerase II TFIIH. Cell 82: 555-564.
4. Iyer, N., et al. 1996. Interactions involving the human RNA polymerase II transcription/nucleotide excision repair complex TFIIH, the nucleotide excision repair protein XPG, and Cockayne syndrome group B (CSB) protein. Biochemistry 35: 2157-2167.
5. Van Gool, A.J., et al. 1997. The Cockayne syndrome B protein, involved in transcription-coupled DNA repair, resides in an RNA polymerase II-containing complex. EMBO J. 16: 5955-5965.
6. Tantin, D. 1998. RNA polymerase II elongation complexes containing the Cockayne syndrome group B protein interact with a molecular complex containing the transcription factor IIH components xeroderma pigmentosum B and p62. J. Biol. Chem. 273: 27794-27799.

CHROMOSOMAL LOCATION

Genetic locus: Ercc8 (mouse) mapping to 13 D2.1.

PRODUCT

CSA 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 CSA shRNA Plasmid (m): sc-37793-SH and CSA shRNA (m) Lentiviral Particles: sc-37793-V as alternate gene silencing products.

For independent verification of CSA (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-37793A, sc-37793B and sc-37793C.

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

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

CSA (D-2): sc-376981 is recommended as a control antibody for monitoring of CSA 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 CSA gene expression knockdown using RT-PCR Primer: CSA (m)-PR: sc-37793-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.