SANTA CRUZ BIOTECHNOLOGY, INC.

Ku86 siRNA (m): sc-35765

BACKGROUND

The Ku protein is localized in the nucleus and is composed of subunits referred to as Ku70 (or p70) and Ku86 or (p86). Ku was first described as an autoantigen to which antibodies were produced in a patient with scleroderma polymyositis overlap syndrome, and was later found in the sera of patients with other rheumatic diseases. Both subunits of the Ku protein have been cloned, and a number of functions have been proposed for Ku, including cell signaling, DNA replication and transcriptional activation. Ku is involved in Pol II-directed transcription by virtue of its DNA binding activity, serving as the regulatory component of the DNA-associated protein kinase that phosphorylates Pol II and transcription factor Sp. Ku proteins also activate transcription from the U1 small nuclear RNA and the human transferrin receptor gene promoters. A Ku-related protein designated the enhancer 1 binding factor (E1BF), composed of two subunits, has been identified as a positive regulator of RNA polymerase I transcription initiation.

REFERENCES

1. Mimori, T., et al. 1981. Characterization of a high molecular weight acidic nuclear protein recognized by autoantibodies in sera from patients with polymyositis-scleroderma overlap. J. Clin. Invest. 68: 611-620.
2. Mimori, T., et al. 1986. Characterization of the DNA-binding protein antigen Ku recognized by autoantibodies from patients with rheumatic disorders. J. Biol. Chem. 261: 2274-2278.
3. Chan, J.Y.C., et al. 1989. Cloning and characterization of a cDNA that encodes a 70 kDa novel human thyroid autoantigen. J. Biol. Chem. 264: 3651-3654.
4. Reeves, W.H., et al. 1989. Molecular cloning of cDNA encoding the p70 (Ku) lupus autoantigen. J. Biol. Chem. 264: 5047-5052.
5. Yaneva, M., et al. 1989. cDNA-derived amino acid sequence of the 86-kDa subunit of the Ku antigen. J. Biol. Chem. 264: 13407-13411.
6. Prabhakar, B.S., et al. 1990. Cell surface expression of the 70-kD component of Ku, a DNA-binding nuclear antigen. J. Clin. Invest. 86: 1301-1305.
7. Stuiver, M.H, et al. 1990. The autoantigen Ku is indistinguishable from NF IV, a protein forming multimeric protein-DNA complexes. J. Exp. Med. 172: 1049-1054.

CHROMOSOMAL LOCATION

Genetic locus: Xrcc5 (mouse) mapping to 1 C3.

PRODUCT

Ku86 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 Ku86 shRNA Plasmid (m): sc-35765-SH and Ku86 shRNA (m) Lentiviral Particles: sc-35765-V as alternate gene silencing products.

For independent verification of Ku86 (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-35765A, sc-35765B and sc-35765C.

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

Ku86 siRNA (m) is recommended for the inhibition of Ku86 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

Ku86 (B-1): sc-5280 is recommended as a control antibody for monitoring of Ku86 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 Ku86 gene expression knockdown using RT-PCR Primer: Ku86 (m)-PR: sc-35765-PR (20 µl, 348 bp). 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.