SANTA CRUZ BIOTECHNOLOGY, INC.

CBF Consensus and Mutant Oligonucleotides

BACKGROUND

Electrophoretic mobility shift assays (EMSAs), also known as gel shift assays, provide a relatively straightforward and sensitive method for studying binding interactions between transcription factors and consensus DNA binding elements. For such studies, DNA probes are provided as double-stranded oligonucleotides designed with 5' OH blunt ends to facilitate labeling to high specific activity with polynucleotide kinase. These are constructed both with specific DNA binding consensus sequences for various transcription factors and as control or "mutant" probes in which one or more nucleotides mapping within the consensus binding site has been substituted.

REFERENCES

1. Dignam, J.D., et al. 1983. Accurate transcription initiation by RNA polymerase II in a soluble extract from isolated mammalian nuclei. Nucleic Acids Res. 11: 1475-1489.
2. Murre, C., et al. 1991. B cell- and myocyte-specific E2-box-binding factors contain E12/E47-like subunits. Mol. Cell. Biol. 11: 1156-1160.
3. Bi, W., et al. 1997. DNA binding specificity of the CCAAT binding factor CBF/NF-Y. J. Biol. Chem. 272: 26562-26572.

GEL SHIFT ASSAYS

For gel shift analysis, prepare nuclear extracts following the method of Dignam, et al (1).

- **NOTE**: Spin oligonucleotide vial before opening. Product may be lodged in vial cap.
- Label oligonucleotide probe (TransCruz™ Gel Shift Oligonucleotides) with [γ^{32} P]-ATP to 50,000 cpm/ng by using polynucleotide kinase.
- Prepare gel shift reaction buffer as follows: 10 mM Tris (Tris: sc-3715), pH 7.5, 50 mM NaCl (NaCl: sc-29108, 1 mM dithiothreitol (DTT: sc-29089), 1 mM EDTA (EDTA: sc-29092), 5% glycerol (glycerol: sc-29095).
- Prepare 20 µl reaction mixture containing 3-10 µg nuclear extract and 1 µg poly dI-dC in gel shift reaction buffer. Add 0.5 ng labeled oligonucleotide probe and incubate for 20 minutes at room temperature. This constitutes the control sample for detection of DNA-protein complexes (2).
- To detect an antibody supershift or block of the DNA-protein complex, prepare reaction mixture as described above, also adding 1-2 µl of the appropriate TransCruz™ Gel Supershift antibody per 20 µl of reaction volume. Antibody is normally added subsequent to addition of labeled oligonucleotide probe, but result may be improved by adding antibody prior to probe. Incubate at 4° C for 1 hour to overnight, or at room temperature for 15-45 minutes.
- Resolve DNA-protein complexes by electrophoresis (25-35 ma) through a 4% polyacrylamide gel containing 50 mM Tris, pH 7.5, 0.38 M glycine (glycine: sc-29096) and 2 mM EDTA. Dry the gel and visualize by autoradiography.

RESEARCH USE

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

PRODUCT

CBF CONSENSUS OLIGONUCLEOTIDE: sc-2591

- binding site for CBF transcription factor (3)

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5′– AGA  CCG  TAC  GTG  ATT  GGT  TAA  TCT  CTT – 3′
3′– TCT  GGC  ATG  CAC  TAA  CCA  ATT  AGA  GAA – 5′
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CBF MUTANT OLIGONUCLEOTIDE: sc-2592

- identical to sc-2591 with the exception of "TG"→"AA" and "TT"→"GG" substitutions in the flanking sequence; and "TG"→"AC" substitution in the CBF binding site (3)

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5′– AGA  CCG  TAC  GAA  ATA  CGG  GAA  TCT  CTT – 3′
3′– TCT  GGC  ATG  CTT  TAT  GCC  CTT  AGA  GAA – 5′
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SELECT PRODUCT CITATIONS

1. Zhao, J.Q., et al. 2000. Activation of telomerase rna gene promoter activity by NF-Y, Sp1, and the retinoblastoma protein and repression by Sp3. Neoplasia 2: 531-539.
2. Scheef, G., et al. 2002. Transcriptional regulation of porcine endogenous retroviruses released from porcine and infected human cells by heterotrimeric protein complex NF-Y and impact of immunosuppressive drugs. J. Virol. 76: 12553-12563.
3. Louneva, N., et al. 2003. Transcriptional inhibition of type I collagen gene expression in scleroderma fibroblasts by the antineoplastic drug ecteinascidin 743. J. Biol. Chem. 278: 40400-40407.
4. Cicchillitti, L., et al. 2004. B-Myb acts as a repressor of human COL1A1 collagen gene expression by interacting with Sp1 and CBF factors in scleroderma fibroblasts. Biochem. J. 378: 609-616.
5. Woo, C.W.H., et al. 2005. Hyperhomocysteinemia induces hepatic cholesterol biosynthesis and lipid accumulation via activation of transcription factors. Am. J. Physiol. Endocrinol. Metab. 288: E1002-E1010.
6. Ikeda, K., et al. 2006. Transcription factor activating enhancer-binding protein-2β: a negative regulator of adiponectin gene expression. J. Biol. Chem. 281: 31245-31253.
7. Mare, L., et al. 2007. Comparative analysis of retroviral and native promoters driving expression of β1,3-galactosyltransferase β3Gal-T5 in human and mouse tissues. J. Biol. Chem. 282: 49-57.
8. Adams, J.P., et al. 2009. NMDA receptor-independent control of transcription factors and gene expression. Neuroreport 20: 1429-1433.
9. Kushnir, A.S., et al. 2010. Role of nuclear factor Y in stress-induced activation of the herpes simplex virus type 1 ICP0 promoter. J. Virol. 84: 188-200.

STORAGE

Store at -20° C; stable for one year from the date of shipment.

NOTE: Spin oligonucleotide vial before opening. Product may be lodged in vial cap.