SANTA CRUZ BIOTECHNOLOGY, INC.

NF-1 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. Jones, K.A., et al. 1987. A cellular DNA-binding protein that activates eukaryotic transcription and DNA replication. Cell 48: 79-89.
3. 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.

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

NF-1 CONSENSUS OLIGONUCLEOTIDE: sc-2553

- binding site for NF-I (3)

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5′– TTT TGG ATT GAA GCC AAT ATG ATA A – 3′
3′– AAA ACC TAA CTT CGG TTA TAC TAT T – 5′
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NF-1 MUTANT OLIGONUCLEOTIDE: sc-2554

- identical to sc-2553 with the exception of a "GCC"→"TAA" substitution in the NF-I binding motif (3)

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5′– TTT TGG ATT GAA TAA AAT ATG ATA A – 3′
3′– AAA ACC TAA CTT ATT TTA TAC TAT T – 5′
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SELECT PRODUCT CITATIONS

1. Nuthall, H.N., et al. 1999. Analysis of DNase-I-hypersensitive sites at the 3' end of the cystic fibrosis transmembrane conductance regulator gene (CFTR). Biochem. J. 341: 601-611.
2. 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.
3. Saur, D., et al. 2002. Complex regulation of human neuronal nitric-oxide synthase exon 1c gene transcription. Essential role of Sp and ZNF family members of transcription factors. J. Biol. Chem. 277: 25798-25814.
4. McGaha, T.L., et al. 2003. Molecular mechanisms of interleukin-4-induced up-regulation of type I collagen gene expression in murine fibroblasts. Arthritis Rheum. 48: 2275-2284.
5. Wang, W., et al. 2004. A role for nuclear factor I in the intrinsic control of cerebellar granule neuron gene expression. J. Biol. Chem. 279: 53491-53497.
6. Carabana, J., et al. 2005. Regulation of the murine Dδ2 promoter by upstream stimulatory factor 1, Runx1, and c-Myb. J. Immunol. 174: 4144-4152.
7. Maier, E.A., et al. 2006. Temporal regulation of enhancer function in intestinal epithelium: a role for Onecut factors. J. Biol. Chem. 281: 32263-32271.
8. Rodríguez-Rodero, S., et al. 2007. Transcriptional regulation of MICA and MICB: a novel polymorphism in MICB promoter alters transcriptional regulation by Sp1. Eur. J. Immunol. 37: 1938-1953.
9. Roberts, L.E., et al. 2007. PD98059 enhanced Insulin, cytokine, and growth factor activation of xanthine oxidoreductase in epithelial cells involves Stat3 and the glucocorticoid receptor. J. Cell. Biochem. 101: 1567-1587.
10. Liu, B., et al. 2011. IKKα represses a network of inflammation and proliferation pathways and elevates c-Myc antagonists and differentiation in a dose-dependent manner in the skin. Cell Death Differ. 18: 1854-1864.

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.