SANTA CRUZ BIOTECHNOLOGY, INC.

AP-2α 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. Williams, T., et al. 1988. Cloning and expression of AP-2, a cell-type-specific transcription factor that activates inducible enhancer elements. Genes Dev. 2: 1557-1569.
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

AP-2α CONSENSUS OLIGONUCLEOTIDE: sc-2513

- binding site for AP-2 transcription factor (3)

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5′– GAT  CGA  ACT  GAC  CGC  CCG  CGG  CCC  GT – 3′
3′– CTA  GCT  TGA  CTG  GCG  GGC  GCC  GGG  CA – 5′
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AP-2α MUTANT OLIGONUCLEOTIDE: sc-2516

- identical to sc-2513 with the exception of a "CC"→"TT" substitution in the AP-2 DNA binding motif (3)

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5′– GAT  CGA  ACT  GAC  CGC  TTG  CGG  CCC  GT – 3′
3′– CTA  GCT  TGA  CTG  GCG  AAC  GCC  GGG  CA – 5′
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SELECT PRODUCT CITATIONS

1. Marriott, S., et al. 1996. Activation of the HTLV-I long terminal repeat by the hepatitis B virus X protein. Virology 224: 206-213.
2. Jiang, M., et al. 1998. Derepression of the C/EBPα gene during adipogenesis: identification of AP-2α as a repressor. Proc. Natl. Acad. Sci. USA 95: 3467-3471.
3. Lukiw, W.J., et al. 1998. Budesonide epimer R or dexamethasone selectively inhibit platelet-activating factor-induced or interleukin-1β-induced DNA binding activity of *cis*-acting transcription factors and cyclooxygenase-2 gene expression in human epidermal keratinocytes. Proc. Natl. Acad. Sci. USA 95: 3914-3919.
4. Masaki, S., et al. 1998. Identification and functional analysis of the mouse lens filensin gene promoter. Gene 214: 77-86.
5. Allgayer, H., et al. 1999. Transactivation of the urokinase-type plasminogen activator receptor gene through a novel promoter motif bound with an activator protein-2α-related factor. J. Biol. Chem. 274: 4702-4714.
6. Lee, Y.W., et al. 2001. Interleukin 4 induces transcription of the 15-lipoxygenase I gene in human endothelial cells. J. Lipid Res. 42: 783-791.
7. 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.
8. Liang, S.X. and Richardson, D.R. 2003. The effect of potent iron chelators on the regulation of p53: examination of the expression, localization and DNA-binding activity of p53 and the transactivation of WAF1. Carcinogenesis 24: 1601-1614.
9. Fujimori, K., et al. 2003. Regulation of lipocalin-type prostaglandin D synthase gene expression by Hes-1 through E-box and interleukin-1 β via two NFκB elements in rat leptomeningeal cells. J. Biol. Chem. 278: 6018-6026.
10. Li, M. and Kellems, R.E. 2003. Sp1 and Sp3 are important regulators of AP-2γ gene transcription. Biol. Reprod. 69: 1220-1230.

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.