SANTA CRUZ BIOTECHNOLOGY, INC.

AP-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. Lee, W., et al. 1987. Purified transcription factor AP-1 interacts with TPA-inducible enhancer elements. Cell 49: 741-752.
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-1 CONSENSUS OLIGONUCLEOTIDE: sc-2501

- binding site for AP-1 c-Jun homodimer and Jun/Fos heterodimeric complexes (3)

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5' - CGC  TTG  ATG  ACT  CAG  CCG  GAA - 3'
3' - GCG  AAC  TAC  TGA  GTC  GGC  CTT - 5'
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AP-1 MUTANT OLIGONUCLEOTIDE: sc-2514

- identical to sc-2501 with the exception of a "CA"→"TG" substitution in the AP-1 binding motif (3)

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5' - CGC  TTG  ATG  ACT  TGG  CCG  GAA - 3'
3' - GCG  AAC  TAC  TGA  ACC  GGC  CTT - 5'
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SELECT PRODUCT CITATIONS

1. Azzoni, L., et al. 1996. IL-12-induced activation of NK and T cells occurs in the absence of immediate-early activation gene expression. J. Immunol. 157: 3235-3241.
2. Wang, B.W., et al. 2003. Induction of matrix metalloproteinases-14 and -2 by cyclical mechanical stretch is mediated by tumor necrosis factor-α in cultured human umbilical vein endothelial cells. Cardiovasc. Res. 59: 460-469.
3. Abboushi, N., et al. 2004. Ceramide inhibits IL-2 production by preventing protein kinase C-dependent NFκB activation: possible role in protein kinase C θ regulation. J. Immunol. 173: 3193-3200.
4. Quan, T., et al. 2005. Ultraviolet irradiation induces Smad7 via induction of transcription factor AP-1 in human skin fibroblasts. J. Biol. Chem. 280: 8079-8085.
5. Jia, Q., et al. 2006. Docosahexaenoic acid consumption inhibits deoxynivalenol-induced CREB/ATF1 activation and IL-6 gene transcription in mouse macrophages. J. Nutr. 136: 366-372.
6. Zhang, J.Y., et al. 2008. The JNK/AP1/ATF2 pathway is involved in H_2O_2-induced acetylcholinesterase expression during apoptosis. Cell. Mol. Life Sci. 65: 1435-1445.
7. Shyu, K.G., et al. 2009. Mechanism of inhibitory effect of atorvastatin on resistin expression induced by tumor necrosis factor-α in macrophages. J. Biomed. Sci. 16: 50.
8. Chusid, L.A., et al. 2010. Transcriptional control of cytokine release from monocytes of the newborn: effects of endogenous and exogenous interleukin-10 versus dexamethasone. Neonatology 97: 108-116.
9. Audard, V., et al. 2012. Upregulation of nuclear factor-related κ B suggests a disorder of transcriptional regulation in minimal change nephrotic syndrome. PLoS ONE 7: e30523.

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.