

C/EBP 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.

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 [³²P]-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.

STORAGE

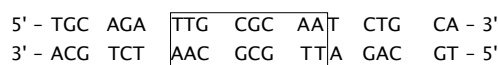
Store at -20° C; stable for one year from the date of shipment.

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

PRODUCT

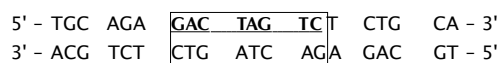
C/EBP CONSENSUS OLIGONUCLEOTIDE: sc-2525

- binding site for CCAAT enhancer binding proteins (3)



C/EBP MUTANT OLIGONUCLEOTIDE: sc-2526

- identical to sc-2525 with the exception of an eight base pair substitution in the binding motif (3)



SELECT PRODUCT CITATIONS

1. Jiang, J.G., et al. 1997. A novel transcriptional regulatory region within the core promoter of the hepatocyte growth factor gene is responsible for its inducibility by cytokines via the C/EBP family of transcription factors. *Mol. Cell. Biol.* 17: 5758-5770.
2. Popik, W., et al. 1998. Binding of human immunodeficiency virus type 1 to CD4 and CXCR4 receptors differentially regulates expression of inflammatory genes and activates the MEK/ERK signaling pathway. *J. Virol.* 72: 6406-6413.
3. 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.
4. Fessele, S., et al. 2001. Molecular and in silico characterization of a promoter module and C/EBP element that mediate LPS-induced RANTES/CCL5 expression in monocytic cells. *FASEB J.* 15: 577-579.
5. Dasgupta, S., et al. 2003. Role of very-late antigen-4 (VLA-4) in myelin basic protein-primed T cell contact-induced expression of proinflammatory cytokines in microglial cells. *J. Biol. Chem.* 278: 22424-22431.
6. Afink, G., et al. 2004. C/EBP is an essential component of PDGFRA transcription in MG-63 cells. *Biochem. Biophys. Res. Commun.* 315: 313-318.
7. Jia, Q., et al. 2006. Docosahexaenoic acid consumption inhibits deoxyribose-induced CREB/ATF1 activation and IL-6 gene transcription in mouse macrophages. *J. Nutr.* 136: 366-372.
8. Byun, S.J., et al. 2007. IFN-γ upregulates expression of the mouse complement C1rA gene in keratinocytes via IFN-regulatory factor-1. *J. Invest. Dermatol.* 127: 1187-1196.
9. Wei, W., et al. 2007. Treatment of cultured myotubes with the proteasome inhibitor β-lactone increases the expression of the transcription factor C/EBPβ. *Am. J. Physiol. Cell Physiol.* 292: C216-C226.
10. Huber, N.L., et al. 2010. Remote thermal injury increases LPS-induced intestinal IL-6 production. *J. Surg. Res.* 160: 190-195.

RESEARCH USE

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