

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. *Nucl. 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. Mahoney, C.W., et al. 1992. Phosphorylation of CCAAT-enhancer binding protein by protein kinase C attenuates site selective DNA binding. *J. Biol. Chem.* 267: 19396-19403.

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 -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 μ l reaction mixture containing 3-10 μ g nuclear extract and 1 μ g 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 μ l of the appropriate TransCruz™ Gel Supershift antibody per 20 μ l 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.

PRODUCT

C/EBP CONSENSUS OLIGONUCLEOTIDE: sc-2525

- binding site for CCAAT enhancer binding proteins (3)

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5' - TGC AGA TTG CGC AAT CTG CA - 3'
3' - ACG TCT AAC GCG TTA GAC GT - 5'
  
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C/EBP MUTANT OLIGONUCLEOTIDE: sc-2526

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

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5' - TGC AGA GAC TAG TCT CTG CA - 3'
3' - ACG TCT CTG ATC AGA GAC GT - 5'
  
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SELECT PRODUCT CITATIONS

1. Ortiz, B., et al. 1996. Kinetics of transcription factors regulating the RANTES chemokine gene reveal a developmental switch in nuclear events during T lymphocyte maturation. *Mol. Cell. Biol.* 16: 202-210.
2. 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.
3. Basso, J., et al. 2000. Directed mutation of the basic domain of v-Jun alters DNA binding specificity and abolishes its oncogenic activity in chicken embryo fibroblasts. *Oncogene* 19: 4876-85.
4. Harris, V., et al. 2001. Serum induction of the fibroblast growth factor-binding protein (FGF-BP) is mediated through ERK and p38 MAP kinase activation and C/EBP-regulated transcription. *Oncogene* 20: 1730-1738.
5. Dumais, N., et al. 2002. Prostaglandin E(2)-mediated activation of HIV-1 long terminal repeat transcription in human T cells necessitates CCAAT/enhancer binding protein (C/EBP) binding sites in addition to cooperative interactions between C/EBP β and cyclic adenosine 5'-monophosphate response element binding protein. *J. Immunol.* 168: 274-282.
6. Haan, C., et al. 2002. Structural requirements of the interleukin-6 signal transducer gp130 for its interaction with Janus kinase 1: the receptor is crucial for kinase activation. *Biochem. J.* 361: 105-111.
7. Wu, J., et al. 2003. A novel polymorphic CAAT/enhancer-binding protein β element in the FasL gene promoter alters Fas ligand expression: a candidate background gene in African American systemic lupus erythematosus patients. *J. Immunol.* 170: 132-138.

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.

RESEARCH USE

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

C/EBP Consensus and Mutant Oligonucleotide Agarose Conjugates

PRODUCT

Transcription factor consensus gel shift oligonucleotides containing specific consensus sequences and mutant control oligonucleotides are provided as agarose conjugates for use in purifying or enriching for specific transcription factors. TransCruz™ Oligonucleotide Agarose Conjugates are provided as 15 µg double-stranded oligonucleotide in 0.25 ml packed beads (1.0 ml total volume). Provides sufficient reagent for 10 assays.

C/EBP CONSENSUS OLIGONUCLEOTIDE: sc-2525 AC

- binding site for CCAAT enhancer binding proteins (3)

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5' - TGC AGA TTT CGC AAT CTG CA - 3'
3' - ACG TCT AAC GCG TTA GAC GT - 5'
  
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C/EBP MUTANT OLIGONUCLEOTIDE: sc-2526 AC

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

```

5' - TGC AGA GAC TAG TCT CTG CA - 3'
3' - ACG TCT CTG ATC AGA GAC GT - 5'
  
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REFERENCES

- Dignam, J.D., et al. 1983. Accurate transcription initiation by RNA polymerase II in a soluble extract from isolated mammalian nuclei. *Nucl. Acids Res.* 11: 1475-1489.
- 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.
- Mahoney, C.W., et al. 1992. Phosphorylation of CCAAT-enhancer binding protein by protein kinase C attenuates site selective DNA binding. *J. Biol. Chem.* 267: 19396-19403.

PREPARATION OF SOLUTIONS

- Binding buffer: 10 mM Tris, pH 7.5; 50 mM NaCl; 1 mM DTT; 1 mM EDTA; 5% glycerol; 1 µg/ml poly dI-dC.
- Elution buffer: Same as binding buffer, but increase NaCl concentration to 150 mM.

PROCEDURE

- Thoroughly mix oligonucleotide agarose conjugate slurry. Aliquot 100 µl slurry (containing 25 µl beads) into 1.5 ml microcentrifuge tube. To pellet beads, centrifuge at 12,000 rpm for 3-5 minutes in microcentrifuge at 4° C. Aspirate supernatant and wash pellet 3 times as follows: add 1 ml binding buffer, resuspend beads and centrifuge at 12,000 rpm for 3-5 minutes in microcentrifuge at 4° C, aspirating supernatant after each wash.
- To the washed agarose pellet, add 250-1000 µg nuclear extract or whole cell lysate (preferably <200 µl in volume). Add sufficient binding buffer to bring total volume to 500 µl. (If a large volume of extract/lysate is used, adjust final NaCl concentration to approximately 50 mM. Alternatively, extract/lysate can be prepared in binding buffer.)

- Incubate with rotation for 2 hours at room temperature or overnight at 4° C. Centrifuge at 12,000 rpm for 3-5 minutes in microcentrifuge at 4° C and aspirate supernatant. Wash pellet 3 times with binding buffer as described above.
- To elute protein from washed beads, add 250 µl elution buffer and incubate for 30 minutes with rotation at room temperature. Centrifuge at 12,000 rpm for 3-5 minutes at 4° C to pellet beads.
- Carefully collect supernatant; this is the protein sample. If desired, concentrate protein sample using a commercially available micro-concentrator.
- To 10-25 µl concentrated sample, add an equal volume of SDS-PAGE electrophoresis sample buffer. Boil for 90 seconds.
- Analyze by Western blot analysis (loading up to 20 µl per lane) or other suitable research application according to Santa Cruz Biotechnology, Inc. research applications protocols.

ALTERNATE PROCEDURE

- Complete preparation and incubation of sample as in steps 1-3 above.
- Directly add 20-50 µl SDS-PAGE electrophoresis sample buffer to the washed pellet. Boil for 90 seconds. Centrifuge at 12,000 rpm for 3-5 minutes in microcentrifuge at 4° C.
- Analyze supernatant by Western blot analysis as in final step above.

SELECT PRODUCT CITATIONS

- Ortiz, B., et al. 1996. Kinetics of transcription factors regulating the RANTES chemokine gene reveal a developmental switch in nuclear events during T lymphocyte maturation. *Mol. Cell. Biol.* 16: 202-210.
- Tezuka, K.I., et al. 1996. Stimulation of mouse osteopontin promoter by v-Src is mediated by a CCAAT box-binding factor. *J. Biol. Chem.* 271: 22713-22717.
- Doyle, G., et al. 1997. Transcriptional induction of collagenase-1 in differentiated monocyte-like (U937) cells is regulated by AP-1 and an upstream C/EBP β site. *J. Biol. Chem.* 272: 11840-11849.
- 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.
- Yukawa, K., et al. 1998. Expressions of CCAAT/enhancer-binding proteins and their activities are intensified by cAMP signaling as well as Ca²⁺/Calmodulin kinases activation in hippocampal neurons. *J. Biol. Chem.* 273: 31345-31351.

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