SANTA CRUZ BIOTECHNOLOGY, INC.

Oct-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. 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. Scheidereit, C., et al. 1988. A human lymphoid-specific transcription factor that activates immunoglobulin genes is a homeobox protein. Nature 336: 551-557.

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.

PRODUCT

Oct-1 CONSENSUS OLIGONUCLEOTIDE: sc-2506

- binding site for Oct family homeodomain transcription factors (3)

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5'— TGT  CGA  ATG  CAA  AT C  ACT  AGA  A — 3'
3'— ACA  GCT  TAC  GTT  TA G  TGA  TCT  T — 5'
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Oct-1 MUTANT OLIGONUCLEOTIDE: sc-2515

- identical to sc-2506 with the exception of an "AT"→"GC" substitution in the Oct DNA binding motif (3)

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5'— TGT  CGA  ATG  CAA  GC C  ACT  AGA  A — 3'
3'— ACA  GCT  TAC  GTT  CG G  TGA  TCT  T — 5'
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SELECT PRODUCT CITATIONS

1. Natarajan, K., et al. 1996. Caffeic Acid phenethyl ester is a potent and specific inhibitor of activation of nuclear transcription factor NFκB. Proc. Natl. Acad. Sci. USA 93: 9090-9095.
2. Breithaupt, T.B., et al. 1996. The suppression of T cell function and NFκB expression by Serine protease inhibitors is blocked by N-acetylcysteine. Cell. Immunol. 173: 124-130.
3. Lei, Z., et al. 1997. *Cis*-acting elements and transacting proteins in the transcriptional inhibition of gonadotropin releasing hormaone gene by human chorionic gonadotropin in immortalized hypothalamic GT-7 neurons. J. Biol. Chem. 272: 14365-14371.
4. Zhu, Y.X., et al. 1997. Critical cytoplasmic domains of human interleukin-9 receptor a chain of interleukin-9 mediated cell proliferation and signal transduction. J. Biol. Chem. 272: 21334-21340.
5. Wu, G.D., et al. 1997. Oct-1 and CCAAT/enhancer-binding protein (C/EBP) bind to overlapping elements within the interleukin-8 promoter. The role of Oct-1 as a transcriptional repressor. J. Biol. Chem. 272: 2396-2403.
6. Cui, H., et al. 1997. Proteasome regulation of activation induced T cell death. Proc. Natl. Acad. Sci. USA 94: 7515-7520.
7. 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.
8. Srivastava, R.K., et al. 1998. The RIIb regulatory subunit of protein kinase A binds to cAMP response element: An alternative cAMP signaling pathway. Proc. Natl. Acad. Sci. USA 95: 6687-6692.

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.