SANTA CRUZ BIOTECHNOLOGY, INC.

TFIID 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. Locker, J. and Buzard, G. 1990. A dictionary of transcription control sequences. DNA Seq. 1: 3-11.
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.

PROTOCOLS

See our web site at www.scbt.com for detailed protocols and support products.

PRODUCT

TFIID CONSENSUS OLIGONUCLEOTIDE: sc-2512

- binding sequence for the TFIID basal transcription factor (3)

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5′– GCA  GAG  CA T  ATA  AAA  TGA  GGT  AGG  A –3′
3′– CGT  CTC  GT A  TAT  TTT  ACT  CCA  TCC  T –5′
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TFIID MUTANT OLIGONUCLEOTIDE: sc-2518

- identical to sc-2512 with the exception of a "TA"→"GC" substitution in the "TATA" box sequence (3)

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5′– GCA  GAG  CA G  CTA  AAA  TGA  GGT  AGG  A –3′
3′– CGT  CTC  GT C  GAT  TTT  ACT  CCA  TCC  T –5′
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SELECT PRODUCT CITATIONS

1. Carter, A.B., et al. 1999. The p38 mitogen-activated protein kinase is required for NFκB-dependent gene expression. The role of TATA-binding protein (TBP). J. Biol. Chem. 274: 30858-30863.
2. McNulty, S.E., et al. 2001. RelA, p50 and inhibitor of κBα are elevated in human metastatic melanoma cells and respond aberrantly to ultraviolet light B. Pigment Cell Res. 14: 456-465.
3. Chew, L.J., et al. 2001. Characterization of the rat GRIK5 kainate receptor subunit gene promoter and its intragenic regions involved in neural cell specificity. J. Biol. Chem. 276: 42162-42171.
4. Boyd, J.M., et al. 2002. Adenovirus E1A N-terminal amino acid sequence requirements for repression of transcription *in vitro* and *in vivo* correlate with those required for E1A interference with TBP-TATA complex formation. J. Virol. 76: 1461-1474.
5. Loewenstein, P.M., et al. 2006. Mutational and functional analysis of an essential subdomain of the adenovirus E1A N-terminal transcription repression domain. Virology 351: 312-321.
6. Dong, S., et al. 2006. NF-Y and Sp1/Sp3 are involved in the transcriptional regulation of the peptidylarginine deiminase type III gene (PADI3) in human keratinocytes. Biochem. J. 397: 449-459.
7. Cloutier, A., et al. 2007. Differential involvement of NFκB and MAP kinase pathways in the generation of inflammatory cytokines by human neutrophils. J. Leukoc. Biol. 81: 567-577.
8. Hohmura, K.I., et al. 2013. Perturbation of discrete sites on a single protein domain with RNA aptamers: targeting of different sides of the TATA-binding protein (TBP). Biosci. Biotechnol. Biochem. 77: 1739-1746.

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.