SANTA CRUZ BIOTECHNOLOGY, INC.

Ets Family 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.
3. Scott, G.K., et al. 1994. Binding of an Ets-related protein within the DNase I hypersensitive site of the HER2/Neu promoter in human breast cancer cells. J. Biol. Chem. 269: 19848-19858.

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

Ets CONSENSUS OLIGONUCLEOTIDE: sc-2549

- binding site for PU.1 and GABPα transcription factors (3)

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5′— GGG  CTG  CTT  GAG  GAA  GTA  TAA  GAA  T — 3′
3′— CCC  GAC  GAA  CTC  CTT  CAT  ATT  CTT  A — 5′
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Ets MUTANT OLIGONUCLEOTIDE: sc-2550

- identical to sc-2549 with the exception of a "GAA"→"AGA" substitution in the Ets binding motif (3)

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5′— GGG  CTG  CTT  GAG  AGA  GTA  TAA  GAA  T — 3′
3′— CCC  GAC  GAA  CTC  TCT  CAT  ATT  CTT  A — 5′
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SELECT PRODUCT CITATIONS

1. Hoare, S., et al. 1999. Identification of a GABP α/β binding site involved in the induction of oxytocin receptor gene expression in human breast cells, potentiation by c-Fos/c-Jun. Endocrinology 140: 2268-2279.
2. Nguyen, H.T., et al. 2000. Cyclic stretch activates p38 SAPK2-, ErbB2-, and AT1-dependent signaling in bladder smooth muscle cells. Am. J. Physiol., Cell Physiol. 279: C1155-C1167.
3. Furihata, K. and Kunicki, T.J. 2002. Characterization of human glycoprotein VI gene 5' regulatory and promoter regions. Arterioscler. Thromb. Vasc. Biol. 22: 1733-1739.
4. Gordon, S.J., et al. 2003. Yin Yang 1 is a lipopolysaccharide-inducible activator of the murine 3' Igh enhancer, Hs3. J. Immunol. 170: 5549-5557.
5. Ko, M., et al. 2004. T cell receptor signaling inhibits glucocorticoid-induced apoptosis by repressing the SRG3 expression via ras activation. J. Biol. Chem. 279: 21903-21915.
6. Dobretsova, A., et al. 2004. Potentiation of myelin proteolipid protein (Plp) gene expression is mediated through AP-1-like binding sites. J. Neurochem. 90: 1500-1510.
7. Gonsky, R., et al. 2006. An IFNG SNP with an estrogen-like response element selectively enhances promoter expression in peripheral but not lamina propria T cells. Genes Immun. 7: 342-351.
8. Mare, L., et al. 2007. Comparative analysis of retroviral and native promoters driving expression of β1,3-galactosyltransferase β3Gal-T5 in human and mouse tissues. J. Biol. Chem. 282: 49-57.
9. Crotti, T.N., et al. 2008. PU.1 and NFATc1 mediate osteoclastic induction of the mouse β3 Integrin promoter. J. Cell. Physiol. 215: 636-644.
10. Bristol, J.A., et al. 2018. A cancer-associated Epstein-Barr virus BZLF1 promoter variant enhances lytic infection. PLoS Pathog. 14: e1007179.

STORAGE

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

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