SANTA CRUZ BIOTECHNOLOGY, INC.

YY1 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. Hariharan, N., et al. 1991. Delta, a transcription factor that binds to downstream elements in several polymerase II promoters, is a functionally versatile zinc finger protein. Proc. Natl. Acad. Sci. USA 88: 9799-9803.

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

YY1 CONSENSUS OLIGONUCLEOTIDE: sc-2533

- binding site for YY1 (NF-E1 or NF-δ) transcription factor (3)

5' - CGC TCC CCG GCC ATC TTG GCG GCT GGT - 3'
3' - GCG AGG GGC CGG TAG AAC CGC CGA CCA - 5'

YY1 MUTANT OLIGONUCLEOTIDE: sc-2534

- identical to sc-2533 with the exception of "C"→"G" and "GCC"→"ATT" substitutions in the DNA binding region (3)

5' - CGC TCC **G**CG **ATT** ATC TTG GCG GCT GGT - 3'
3' - GCG AGG CGC TAA TAG AAC CGC CGA CCA - 5'

SELECT PRODUCT CITATIONS

1. Breen, G.A., et al. 1996. Nuclear factor YY1 activates the mammalian F0F1 ATP synthase α-subunit gene. Gene Expr. 5: 181-191.
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. Brown, J.L., et al. 1998. The *Drosophila* Polycomb group gene pleiohomeotic encodes a DNA binding protein with homology to the transcription factor YY1. Mol. Cell 1: 1057-1064.
4. Riquet, F.B., et al. 2001. YY1 is a positive regulator of transcription of the Col1a1 gene. J. Biol. Chem. 276: 38665-38672.
5. Saur, D., et al. 2002. Complex regulation of human neuronal nitric-oxide synthase exon 1c gene transcription. Essential role of Sp and ZNF family members of transcription factors. J. Biol. Chem. 277: 25798.
6. Maier, E.A., et al. 2005. Cdx binding determines the timing of enhancer activation in postnatal duodenum. J. Biol. Chem. 280: 13195-13202.
7. Ai, W., et al. 2006. Yin Yang 1 (YY1) represses histidine decarboxylase gene expression with SREBP-1a in part through an upstream Sp1 site. Am. J. Physiol. Gastrointest. Liver Physiol. 290: G1096-G1104.
8. Gao, W., et al. 2009. Calcium signaling-induced Smad3 nuclear accumulation induces acetylcholinesterase transcription in apoptotic HeLa cells. Cell. Mol. Life Sci. 66: 2181-2193.
9. Li, J.R., et al. 2011. A common promoter variant of TBX21 is associated with allele specific binding to Yin-Yang 1 and reduced gene expression. Scand. J. Immunol. 73: 449-458.
10. Han, S.S., et al. 2014. Piperlongumine inhibits the proliferation and survival of B-cell acute lymphoblastic leukemia cell lines irrespective of glucocorticoid resistance. Biochem. Biophys. Res. Commun. 452: 669-675.

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.