SANTA CRUZ BIOTECHNOLOGY, INC.

Sp1 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. Kriwac ki, R.W., et al. 1992. Sequence-specific recognition of DNA by zinc-finger peptides derived from the transcription factor Sp1. Proc. Natl. Acad. Sci. USA 89: 9759-9763.

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

Sp1 CONSENSUS OLIGONUCLEOTIDE: sc-2502

- binding site for Sp1 transcription factor (3)

5' – ATT	CGA	TCG	GGG	CGG	GGC	GAG	C – 3'
3' – TAA	GCT	AGC	CCC	GCC	CCG	CTC	G – 5'

Sp1 MUTANT OLIGONUCLEOTIDE: sc-2503

- identical to sc-2502 with the exception of a "GG"→"TT" substitution in the Sp1 binding motif (3)

5' – ATT	CGA	TCG	GTT	CGG	GGC	GAG	C – 3'
3' – TAA	GCT	AGC	CAA	GCC	CCG	CTC	G – 5'

SELECT PRODUCT CITATIONS

1. Kumar, A.P., et al. 1997. Transcription factor Sp3 antagonizes activation of the ornithine decarboxylase promotor by Sp1. Nucleic Acids Res. 25: 2012-2019.
2. Jiang, J.G., et al. 1997. Transcriptional regulation of the hepatocyte growth factor (HGF) gene by the Sp family of transcription factors. Oncogene 14: 3039-3049.
3. 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: 25798-25814.
4. Tan, L., et al. 2003. Egr-1 mediates transcriptional repression of COL2A1 promoter activity by interleukin-1β. J. Biol. Chem. 278: 17688-17700.
5. Steinke, J.W., et al. 2004. Functional analysis of -571 IL-10 promoter polymorphism reveals a repressor element controlled by sp1. J. Immunol. 173: 3215-3222.
6. Kumbrink, J., et al. 2005. Egr-1 induces the expression of its corepressor nab2 by activation of the nab2 promoter thereby establishing a negative feedback loop. J. Biol. Chem. 280: 42785-42793.
7. Akimzhanov, A., et al. 2008. Epigenetic changes and suppression of the nuclear factor of activated T cell 1 (NFATC1) promoter in human lymphomas with defects in immunoreceptor signaling. Am. J. Pathol. 172: 215-224.
8. Goldman, S., et al. 2009. Mechanisms of matrix metalloproteinase-2 (mmp-2) transcriptional repression by progesterone in jar choriocarcinoma cells. Reprod. Biol. Endocrinol. 7: 41.

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.

PROTOCOLS

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