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. Kadonaga, J.T., et al. 1988. Distinct regions of Sp1 modulate DNA binding and transcriptional activation. Science 242: 1566.

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	TC	G	GGG	CGG	GGC		GAG	C – 3'
3' – TAA	GCT	AG	C	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	TC	G	GTT	CGG	GGC		GAG	C – 3'
3' – TAA	GCT	AG	C	CAA	GCC	CCG		CTC	G – 5'

SELECT PRODUCT CITATIONS

1. Tamaki, T., et al. 1995. Characterization of a GC-rich region containing Sp1 binding site(s) as a constitutive responsive element of the α 2(I) collagen gene in human fibroblasts. J. Biol. Chem. 270: 4299-4304.
2. Lauth, M., et al. 2000. Elevated perfusion pressure upregulates endothelin-1 and endothelin B receptor expression in the rabbit carotid artery. Hypertension 35: 648-654.
3. Fessele, S., et al. 2001. Molecular and in silico characterization of a promoter module and C/EBP element that mediate LPS-induced RANTES/CCL5 expression in monocytic cells. FASEB J. 15: 577-579.
4. Wada H,, et al. 2002. Calcineurin-GATA-6 pathway is involved in smooth muscle-specific transcription. J. Cell Biol. 156: 983-991.
5. Yu, B., et al. 2003. Stability of the Sp3-DNA complex is promoter-specific: Sp3 efficiently competes with Sp1 for binding to promoters containing multiple Sp-sites. Nucleic Acids Res. 31: 5368-5376.
6. Hirai, M. 2004. FOG-2 competes with GATA-4 for transcriptional coactivator p300 and represses hypertrophic responses in cardiac myocytes. J. Biol. Chem. 279: 37640-37650.
7. Bradbury D., et al. 2005. Vascular endothelial growth factor induction by prostaglandin E2 in human airway smooth muscle cells is mediated by E prostanoid EP2/EP4 receptors and SP-1 transcription factor binding sites. J. Biol. Chem. 280: 29993-30000.
8. Muckenfuss, H., et al. 2007. Sp1 and Sp3 regulate basal transcription of the human APOBEC3G gene. Nucleic Acids Res. 35: 3784-3796.
9. Harju-Baker, S., et al. 2008. Silencing of Aγ-globin gene expression during adult definitive erythropoiesis mediated by GATA-1-FOG-1-Mi2 complex binding at the -566 GATA site. Mol. Cell. Biol. 28: 3101-3113.
10. Linher, K., et al. 2009. An epigenetic mechanism regulates germ cell-specific expression of the porcine deleted in azoospermia-Like (DAZL) gene. Differentiation 77: 335-349.

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.