SANTA CRUZ BIOTECHNOLOGY, INC.

SIE 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. Sadowski, H.B., et al. 1993. A common nuclear signal transduction pathway activated by growth factor cytokine receptors. Science 261: 1739-1744.

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

SIE CONSENSUS OLIGONUCLEOTIDE: sc-2535

- binding site (SIS-inducible element) for SIS-inducible factor (SIF) (3)

5' – GTG	CAT	TTC	CCG	TAA	ATC	TTG	TCT	ACA – 3'
3' – CAC	GTA	AAG	GGC	ATT	TAG	AAC	AGA	TGT – 5'

SIE MUTANT OLIGONUCLEOTIDE: sc-2536

- identical to sc-2535 with the exception of a "TTC"→"CCA" substitution in the DNA binding region (3)

5' – GTG	CAT	CCA	CCG	TAA	ATC	TTG	TCT	ACA – 3'
3' – CAC	GTA	GGT	GGC	ATT	TAG	AAC	AGA	TGT – 5'

SELECT PRODUCT CITATIONS

1. Waxman, D.J., et al. 1995. Intermittent plasma growth hormone triggers tyrosine phosphorylation and nuclear translocation of a liver-expressed, Stat5-related DNA binding protein J. Biol. Chem. 270: 1-9.
2. Wu, Y.Y., et al. 1996. Synergistic induction of neurite outgrowth by nerve growth factor or epidermal growth factor and interleukin-6 in PC12 cells. J. Biol. Chem. 271: 13033-13039.
3. Venema, R.C., et al. 1998. Angiotensin II-induced tyrosine phosphorylation of signal transducers and activators of transcription 1 is regulated by Janus-activated kinase 2 and Fyn kinases and mitogen-activated protein kinase phosphatase. J. Biol. Chem. 273: 30795-30800.
4. Ndubuisi, M.I., et al. 1999. Cellular physiology of Stat3: where's the cytoplasmic monomer? J. Biol. Chem 274: 25499-25509.
5. Park, O.K., et al. 2000. Dimer stability as a determinant of differential DNA bidning activity of Stat3 isoforms. J. Biol. Chem. 275: 32244-32249.
6. Dikdan, G.S., et al. 2004. Role of oxidative stress in the increased activation of signal transducers and activators of transcription-3 in the fatty livers of obese Zucker rats. Surgery 136: 677-685.
7. Shah, M., et al. 2005. Monocrotaline pyrrole-induced endothelial cell megalocytosis involves a Golgi blockade mechanism. Am. J. Physiol. Cell Physiol. 288: C850-C862.
8. Steiner, E., et al. 2006. The major vault protein is responsive to and interferes with interferon-γ-mediated STAT1 signals. J. Cell Sci. 119: 459-469.
9. Nagy, Z.S. and Rui, H. 2006. A preferential role for STAT5, not constitutively active STAT3, in promoting survival of a human lymphoid tumor. J. Immunol. 177: 5032-5040.
10. Bong, Y.S., et al. 2007. EphrinB1 signals from the cell surface to the nucleus by recruitment of STAT3. Proc. Natl. Acad. Sci. USA 104: 17305-17310.

STORAGE

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

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