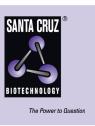
SIE Consensus and Mutant Oligonucleotides



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.
- 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 [γ³² 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 dl-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	тст	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	тст	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.
- Ndubuisi, M.I., et al. 1999. Cellular physiology of Stat3: where's the cytoplasmic monomer? J. Biol. Chem 274: 25499-25509.
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- 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.
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- 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.
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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.