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 4º 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.

PROTOCOLS

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

PRODUCT

SIE CONSENSUS OLIGONUCLEOTIDE: sc-2535

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

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5'— GTG  CAT  TTC  CCG  TAA  ATC  TTG  TCT  ACA —3'
3'— CAC  GTA  AAG  GGC  ATT  TAG  AAC  AGA  TGT —5'
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SIE MUTANT OLIGONUCLEOTIDE: sc-2536

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

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5'— GTG  CAT  CCA  CCG  TAA  ATC  TTG  TCT  ACA —3'
3'— CAC  GTA  GGT  GGC  ATT  TAG  AAC  AGA  TGT —5'
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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. Proposed role as an intracellular regulator of male-specific liver gene transcription. J. Biol. Chem. 270: 13262-13270.
2. Ram, P.A., et al. 1996. Growth hormone activation of Stat1, Stat3, and Stat5 in rat liver. Differential kinetics of hormone desensitization and growth hormone stimulation of both tyrosine phosphorylation and serine/threonine phosphorylation. J. Biol. Chem. 271: 5929-5940.
3. Kodama, H., et al. 1997. Leukemia inhibitory factor, a potent cardiac hypertrophic cytokine, activates the JAK/STAT pathway in rat cardiomyocytes. Circ. Res. 81: 656-663.
4. Kodama, H., et al. 1998. Biphasic activation of the JAK/STAT pathway by Angiotensin II in rat cardiomyocytes. Circ. Res. 82: 244-250.
5. Seto, M., et al. 1998. Effects of prednisolone on glomerular signal transduction cascades in experimental glomerulonephritis. J. Am. Soc. Nephrol. 9: 1367-1376.
6. Wang, Z., et al. 2002. Selective inhibition of Stat3 phosphorylation by sodium salicylate in cardiac fibroblasts. Biochem. Pharmacol. 63: 1197-1207.
7. Olazabal, I.M., et al. 2009. Prolactin's role in the early stages of liver regeneration in rats. J. Cell. Physiol. 219: 626-633.

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.

RESEARCH USE

For research use only, not for use in diagnostic procedures.