SANTA CRUZ BIOTECHNOLOGY, INC.

Stat5/Stat6 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. Hou, J., et al. 1994. An interleukin-4-induced transcription factor: IL-4 Stat. Science 265: 1701-1706.

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.

PROTOCOLS

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

PRODUCT

Stat5/Stat6 CONSENSUS OLIGONUCLEOTIDE: sc-2567

- binding site for Stat5 and Stat6 (3)

```
5′— GTA  TTT  CCC  AGA  AAA  GGA  AC  —3′
3′— CAT  AAA  GGG  TCT  TTT  CCT  TG  —5′
```

Stat5/Stat6 MUTANT OLIGONUCLEOTIDE: sc-2568

- identical to sc-2565 with the exception of a "CCAG"→"GGTT" substitution in the Stat5/Stat6 binding motif (3)

```
5′— GTA  TTT  CGG  TTA  AAA  GGA  AC  —3′
3′— CAT  AAA  GCC  AAT  TTT  CCT  TG  —5′
```

SELECT PRODUCT CITATIONS

1. Lee, Y.W., et al. 2001. Interleukin 4 induces transcription of the 15-lipoxygenase I gene in human endothelial cells. J. Lipid Res. 42: 783-791.
2. Abu-Amer, Y. 2001. IL-4 abrogates osteoclastogenesis through STAT6-dependent inhibition of NF-κB. J. Clin. Invest. 107: 1375-1385.
3. Yuda, H., et al. 2004. Activated protein C inhibits bronchial hyperresponsiveness and Th2 cytokine expression in mice. Blood 103: 2196-2204.
4. Steinke, J.W., et al. 2009. Modulation by aspirin of nuclear phospho-signal transducer and activator of transcription 6 expression: possible role in therapeutic benefit associated with aspirin desensitization. J. Allergy Clin. Immunol. 124: 724-730.e4.

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.