SANTA CRUZ BIOTECHNOLOGY, INC.

NFATc 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. Northrop, J.P., et al. 1994. NFAT components define a family of transcription factors targeted in T cell activation. Nature 369: 497-502.

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

NFATc CONSENSUS OLIGONUCLEOTIDE: sc-2577

- binding site for NFATc (3)

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5′— CGC  CCA  AAG  AGG  AAA  ATT  TGT  TTC  ATA — 3′
3′— GCG  GGT  TTC  TCC  TTT  TAA  ACA  AAG  TAT — 5′
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NFATc MUTANT OLIGONUCLEOTIDE: sc-2578

- identical to sc-2577 with the exception of a "AGG"→"CTT" substitution in the NFATc binding motif (3)

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5′— CGC  CCA  AAG  CTT  AAA  ATT  TGT  TTC  ATA — 3′
3′— GCG  GGT  TTC  GAA  TTT  TAA  ACA  AAG  TAT — 5′
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SELECT PRODUCT CITATIONS

1. Ali, H., et al. 2000. Chemokine production by G protein-coupled receptor activation in a human mast cell line: roles of extracellular signal-regulated kinase and NFAT1. J. Immunol. 165: 7215-7223.
2. Yellaturu, C.R., et al. 2002. A potential role for nuclear factor of activated T-cells in receptor tyrosine kinase and G protein-coupled receptor agonist-induced cell proliferation. Biochem. J. 368: 183-190.
3. Abboushi, N., et al. 2004. Ceramide inhibits IL-2 production by preventing protein kinase C-dependent NFκB activation: possible role in protein kinase C θ regulation. J. Immunol. 173: 3193-3200.
4. Kane, G.C., et al. 2006. KCNJ11 gene knockout of the Kir6.2 KATP channel causes maladaptive remodeling and heart failure in hypertension. Hum. Mol. Genet. 15: 2285-2297.
5. Crotti, T.N., et al. 2006. NFATc1 regulation of the human β3 integrin promoter in osteoclast differentiation. Gene 372: 92-102.
6. Holloway, M.G., et al. 2007. Loss of sexually dimorphic liver gene expression upon hepatocyte-specific deletion of Stat5a-Stat5b locus. Endocrinology 148: 1977-1986.
7. Crotti, T.N., et al. 2008. PU.1 and NFATc1 mediate osteoclastic induction of the mouse β3 integrin promoter. J. Cell. Physiol. 215: 636-644.
8. Kim, B., et al. 2011. Uridine 5'-diphosphate induces chemokine expression in microglia and astrocytes through activation of the P2Y6 receptor. J. Immunol. 186: 3701-3709.
9. Audard, V., et al. 2012. Upregulation of nuclear factor-related κB suggests a disorder of transcriptional regulation in minimal change nephrotic syndrome. PLoS ONE 7: e30523.

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.