SANTA CRUZ BIOTECHNOLOGY, INC.

Stat4 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. Yamamoto, K., et al. 1994. Stat4, a novel γ interferon activation site-binding protein expressed in early myeloid differentiation. Mol. Cell. Biol. 14: 4342-4349.

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

Stat4 CONSENSUS OLIGONUCLEOTIDE: sc-2569

- binding site for Stat4 (3)

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5′– GAG  CCT  GAT  TTC  CCC  GAA  ATG  ATG  AGC  TAG – 3′
3′– CTC  GGA  CTA  AAG  GGG  CTT  TAC  TAC  TCG  ATC – 5′
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Stat4 MUTANT OLIGONUCLEOTIDE: sc-2570

- identical to sc-2569 with the exception of a "CCC"→"TTT" substitution in the Stat4 binding motif (3)

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5′– GAG  CCT  GAT  TTC  TTT  GAA  ATG  ATG  AGC  TAG – 3′
3′– CTC  GGA  CTA  AAG  AAA  CTT  TAC  TAC  TCG  ATC – 5′
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SELECT PRODUCT CITATIONS

1. Ahn, H.J., et al. 1998. Requirement for distinct Janus kinases and STAT proteins in T cell proliferation versus IFN-γ production following IL-12 stimulation. J. Immunol. 161: 5893-5900.
2. Lúdvíksson, B.R., et al. 1999. Role of IL-12 in intrathymic negative selection. J. Immunol. 163: 4349-4359.
3. Dumler, I., et al. 1999. Urokinase induces activation and formation of Stat4 and Stat1-Stat2 complexes in human vascular smooth muscle cells. J. Biol. Chem. 274: 24059-24065.
4. Nakahira, M., et al. 2002. Synergy of IL-12 and IL-18 for IFN-γ gene expression: IL-12-induced Stat4 contributes to IFN-γ promoter activation by up-regulating the binding activity of IL-18-induced activator protein 1. J. Immunol. 168: 1146-1153.
5. Gonsky, R., et al. 2003. CD2 mediates activation of the IFN-γ intronic STAT binding region in mucosal T cells. Eur. J. Immunol. 33: 1152-1162.
6. Schroeter, C.H., et al. 2004. Nuclear factor κB activation in human cord blood mononuclear cells. Pediatr. Res. 56: 212-218.
7. Kremer, M., et al. 2006. Favored T helper 1 response in a mouse model of hepatosteatosis is associated with enhanced T cell-mediated hepatitis. Hepatology 44: 216-227.
8. Sakai, N., et al. 2009. The importance of heterogeneous nuclear ribonucleoprotein K on cytochrome P450 2D2 gene regulation: its binding is reduced in Dark Agouti rats. Drug Metab. Dispos. 37: 1703-1710.
9. Han, S.S., et al. 2014. Piperlongumine inhibits the proliferation and survival of B-cell acute lymphoblastic leukemia cell lines irrespective of glucocorticoid resistance. Biochem. Biophys. Res. Commun. 452: 669-675.

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.

PROTOCOLS

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