SANTA CRUZ BIOTECHNOLOGY, INC.

Stat1 mutant oligonucleotide

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. Mui, A., et al. 1995. Interleukin-3, granulocyte-macrophage colony stimulating factor and interleukin-5 transduce signals through two Stat5 homologs. EMBO J. 14: 1166-1175.

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

Stat1 p84/p91 CONSENSUS OLIGONUCLEOTIDE: sc-2573

- binding site for Stat1 p84/p91 (3)

5' – CAT	GTT	ATG	CAT	ATT	CCT	GTA	AGT	G	–	3'
3' – GTA	CAA	TAC	GTA	TAA	GGA	CAT	TCA	C	–	5'

Stat1 p84/p91 MUTANT OLIGONUCLEOTIDE: sc-2574

- identical to sc-2573 with the exception of a "CCT"→"GGA" substitution in the Stat1 p84/p91 binding motif (3)

5' – CAT	GTT	ATG	CAT	ATT	**GGA**	GTA	AGT	G	–	3'
3' – GTA	CAA	TAC	GTA	TAA	CCT	CAT	TCA	C	–	5'

SELECT PRODUCT CITATIONS

1. Chaturvedi, P., et al. 1997. Abrogation of interleukin-3 dependence of myeloid cells by the v-Src oncogene requires SH2 and SH3 domains which specify activation of Stats. Mol. Cell. Biol. 17: 3295-3304.
2. Osaki, M., et al. 2003. The TATA-containing core promoter of the type II collagen gene (COL2A1) is the target of interferon-γ-mediated inhibition in human chondrocytes: requirement for Stat1 α, Jak1 and Jak2. Biochem. J. 369: 103-115.
3. Naschberger, E., et al. 2004. Nuclear factor-κB motif and interferon-α-stimulated response element co-operate in the activation of guanylate-binding protein-1 expression by inflammatory cytokines in endothelial cells. Biochem. J. 379: 409-420.
4. Fujigaki, H., et al. 2006. The signal transducer and activator of transcription 1α and interferon regulatory factor 1 are not essential for the induction of indoleamine 2,3-dioxygenase by lipopolysaccharide: involvement of p38 mitogen-activated protein kinase and nuclear factor-κB pathways, and synergistic effect of several proinflammatory cytokines. J. Biochem. 139: 655-662.
5. Rigby, R.J., et al. 2007. Suppressor of cytokine signaling 3 (SOCS3) limits damage-induced crypt hyper-proliferation and inflammation-associated tumorigenesis in the colon. Oncogene 26: 4833-4841.
6. Norkina, O., et al. 2008. Acute alcohol intake induces SOCS1 and SOCS3 and inhibits cytokine-induced Stat1 and Stat3 signaling in human monocytes. Alcohol. Clin. Exp. Res. 32: 1565-1573.
7. Strassheim, D., et al. 2009. Prostacyclin inhibits IFN-γ-stimulated cytokine expression by reduced recruitment of CBP/p300 to STAT1 in a SOCS-1-independent manner. J. Immunol. 183: 6981-6988.
8. Saksena, S., et al. 2010. Mechanisms of transcriptional modulation of the human anion exchanger SLC26A3 gene expression by IFN-γ. Am. J. Physiol. Gastrointest. Liver Physiol. 298: G159-G166.

STORAGE

Store at -20° C; stable for one year from the date of shipment.

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