SANTA CRUZ BIOTECHNOLOGY, INC.

Stat3 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. Yu, C.L., et al. 1995. Enhanced DNA-binding activity of a Stat3-related protein in cells transformed by the Src oncoprotein. Science 269: 81-83.

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

Stat3 CONSENSUS OLIGONUCLEOTIDE: sc-2571

- binding site for Stat3 (3)

5' – GAT CCT TCT GGG AAT TCC TAG ATC – 3'
3' – CTA GGA AGA CCC TTA AGG ATC TAG – 5'

Stat3 MUTANT OLIGONUCLEOTIDE: sc-2572

- identical to sc-2571 with the exception of an "AAT"→"CCG" substitution in the Stat3 binding motif (3)

5' – GAT CCT TCT GGG **CCG** TCC TAG ATC – 3'
3' – CTA GGA AGA CCC GGC AGG ATC TAG – 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. Giri, S., et al. 2002. Galactosylsphingosine (psychosine)-induced expression of cytokine-mediated inducible nitric oxide synthases via AP-1 and C/EBP: implications for Krabbe disease. FASEB J. 16: 661-672.
3. Wei, D., et al. 2003. Stat3 activation regulates the expression of vascular endothelial growth factor and human pancreatic cancer angiogenesis and metastasis. Oncogene 22: 319-329.
4. Zancai, P., et al. 2004. Retinoic acid inhibits IL-6-dependent but not constitutive STAT3 activation in Epstein-Barr virus-immortalized B lymphocytes. Int. J. Oncol. 25: 345-355.
5. Kawakami, Y., et al. 2006. Regulation of dendritic cell maturation and function by Bruton's tyrosine kinase via IL-10 and Stat3. Proc. Natl. Acad. Sci. USA 103: 153-158.
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. Ernst, M.B., et al. 2009. Enhanced Stat3 activation in POMC neurons provokes negative feedback inhibition of leptin and Insulin signaling in obesity. J. Neurosci. 29: 11582-11593.
8. Chusid, L.A., et al. 2010. Transcriptional control of cytokine release from monocytes of the newborn: effects of endogenous and exogenous interleukin-10 versus dexamethasone. Neonatology 97: 108-116.
9. Gritsina, G., et al. 2015. Targeted blockade of JAK/STAT3 signaling inhibits ovarian carcinoma growth. Mol. Cancer Ther. 14: 1035-1047.
10. Lu, C., et al. 2019. Type I interferon suppresses tumor growth through activating the STAT3-granzyme B pathway in tumor-infiltrating cytotoxic T lymphocytes. J. Immunother. Cancer 7: 157.

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.