SANTA CRUZ BIOTECHNOLOGY, INC.

# Stat5 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. 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 [$\gamma^{32}$ 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

**Stat5 CONSENSUS OLIGONUCLEOTIDE: sc-2565**

- binding site for Stat5 (3)

```
5′— AGA  TTT  CTA  GGA  ATT  CAA  TCC —3′
3′— TCT  AAA  GAT  CCT  TAA  GTT  AGG —5′
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**Stat5 MUTANT OLIGONUCLEOTIDE: sc-2566**

- identical to sc-2565 with the exception of a "CTAGG"→"AGTTT" substitution in the Stat5 binding motif (3)

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5′— AGA  TTT  AGT  TTA  ATT  CAA  TCC  —3′
3′— TCT  AAA  TCA  AAT  TAA  GTT  AGG  —5′
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## 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. Ishiguro, K. and Sartorelli, A.C. 1997. Clonal variability in β-globin mRNA content in an interleukin-3-dependent bone marrow cell line transfected with the erythropoietin receptor before and after stimulation with erythropoietin. Blood 90: 2273-2281.
3. Tharappel, J.C., et al. 2002. Regulation of cell proliferation, apoptosis, and transcription factor activities during the promotion of liver carcinogenesis by polychlorinated biphenyls. Toxicol. Appl. Pharmacol. 179: 172-184.
4. Jiang, Q., et al. 2004. Distinct regions of the interleukin-7 receptor regulate different Bcl2 family members. Mol. Cell. Biol. 24: 6501-6513.
5. Cao, H., et al. 2006. Novel role for STAT-5B in the regulation of Hsp27-FGF-2 axis facilitating thrombin-induced vascular smooth muscle cell growth and motility. Circ. Res. 98: 913-922.
6. Yumet, G., et al. 2006. Hepatic growth hormone resistance during sepsis is associated with increased suppressors of cytokine signaling expression and impaired growth hormone signaling. Crit. Care Med. 34: 1420-1427.
7. Olazabal, I.M., et al. 2009. Prolactin's role in the early stages of liver regeneration in rats. J. Cell. Physiol. 219: 626-633.


## 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.