

# Stat5/Stat6 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. Hou, J., et al. 1994. An interleukin-4-induced transcription factor: IL-4 Stat. *Science* 265: 1701-1706.

## 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 [ $\gamma$ -<sup>32</sup>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  $\mu$ l reaction mixture containing 3-10  $\mu$ g nuclear extract and 1  $\mu$ g 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  $\mu$ l of the appropriate TransCruz™ Gel Supershift antibody per 20  $\mu$ l 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.

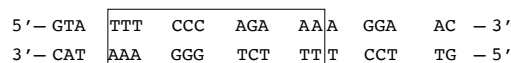
## RESEARCH USE

For research use only, not for use in diagnostic procedures.

## PRODUCT

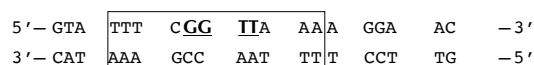
**Stat5/Stat6 CONSENSUS OLIGONUCLEOTIDE: sc-2567**

- binding site for Stat5 and Stat6 (3)



**Stat5/Stat6 MUTANT OLIGONUCLEOTIDE: sc-2568**

- identical to sc-2565 with the exception of a "CCAG" → "GGTT" substitution in the Stat5/Stat6 binding motif (3)



## SELECT PRODUCT CITATIONS

1. Yoshidome, H., et al. 1999. IL-13 activates Stat6 and inhibits liver injury induced by ischemia/reperfusion. *Am. J. Pathol.* 155: 1059-1064.
2. Kato, A., et al. 2000. Regulation of liver inflammatory injury by signal transducer and activator of transcription-6. *Am. J. Pathol.* 157: 297-302.
3. Yamazaki, F., et al. 2002. Overactivation of IL-4-induced activator protein-1 in atopic dermatitis. *J. Dermatol. Sci.* 28: 227-233.
4. Gonsky, R., et al. 2003. CD2 mediates activation of the IFN- $\gamma$  intronic STAT binding region in mucosal T cells. *Eur. J. Immunol.* 33: 1152-1162.
5. Albina, J.E., et al. 2005. Macrophage arginase regulation by CCAAT/enhancer-binding protein  $\beta$ . *Shock* 23: 168-172.
6. Tabata, Y., et al. 2006. Allergy-driven alternative splicing of IL-13 receptor  $\alpha$ 2 yields distinct membrane and soluble forms. *J. Exp. Med.* 177: 7905-7912.
7. Matsui, S., et al. 2006. Glycyrrhizin derivative inhibits eotaxin 1 production via Stat6 in human lung fibroblasts. *Int. Immunopharmacol.* 6: 369-375.
8. Nakahira, M., et al. 2007. Regulation of signal transducer and activator of transcription signaling by the tyrosine phosphatase PTP-BL. *Immunity* 26: 163-176.
9. 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.
10. Datta, R., et al. 2011. PARP-1 deficiency blocks IL-5 expression through calpain-dependent degradation of STAT-6 in a murine asthma model. *Allergy* 66: 853-861.
11. 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.