



# NFATc 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. Northrop, J.P., et al. 1994. NFAT components define a family of transcription factors targeted in T cell activation. *Nature* 369: 497-502.

## 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

### NFATc CONSENSUS OLIGONUCLEOTIDE: sc-2577

- binding site for NFATc (3)

```

5' - CGC CCA AAG AGG AAA ATT TGT TTC ATA - 3'
3' - GCG GGT TTC TCC TTT TAA ACA AAG TAT - 5'
  
```

### NFATc MUTANT OLIGONUCLEOTIDE: sc-2578

- identical to sc-2577 with the exception of a "AGG"→"CTT" substitution in the NFATc binding motif (3)

```

5' - CGC CCA AAG CTT AAA ATT TGT TTC ATA - 3'
3' - GCG GGT TTC GAA TTT TAA ACA AAG TAT - 5'
  
```

## SELECT PRODUCT CITATIONS

1. Ali, H., et al. 2000. Chemokine production by G protein-coupled receptor activation in a human mast cell line: roles of extracellular signal-regulated kinase and NFAT1. *J. Immunol.* 165: 7215-7223.
2. Abboushi, N., et al. 2004. Ceramide inhibits IL-2 production by preventing protein kinase C-dependent NF $\kappa$ B activation: possible role in protein kinase C  $\theta$  regulation. *J. Immunol.* 173: 3193-3200.
3. Kane, G.C., et al. 2006. KCNJ11 gene knockout of the Kir6.2 KATP channel causes maladaptive remodeling and heart failure in hypertension. *Hum. Mol. Genet.* 15: 2285-2297.
4. Crotti, T.N., et al. 2006. NFATc1 regulation of the human  $\beta$ 3 integrin promoter in osteoclast differentiation. *Gene* 372: 92-102.
5. Holloway, M.G., et al. 2007. Loss of sexually dimorphic liver gene expression upon hepatocyte-specific deletion of Stat5a-Stat5b locus. *Endocrinology* 148: 1977-1986.
6. Crotti, T.N., et al. 2008. PU.1 and NFATc1 mediate osteoclastic induction of the mouse  $\beta$ 3 integrin promoter. *J. Cell. Physiol.* 215: 636-644.
7. Kim, B., et al. 2011. Uridine 5'-diphosphate induces chemokine expression in microglia and astrocytes through activation of the P2Y6 receptor. *J. Immunol.* 186: 3701-3709.
8. Audard, V., et al. 2012. Upregulation of nuclear factor-related  $\kappa$ B suggests a disorder of transcriptional regulation in minimal change nephrotic syndrome. *PLoS ONE* 7: e30523.
9. Zhai, Q., et al. 2020. CircRNA hsa\_circ\_0008500 acts as a miR-1301-3p sponge to promote osteoblast mineralization by upregulating PADI4. *Front. Cell Dev. Biol.* 8: 602731.
10. Neuschäfer-Rube, F., et al. 2023. LDL-dependent regulation of TNF $\alpha$ /PGE<sub>2</sub> induced COX-2/mPGES-1 expression in human macrophage cell lines. *Inflammation* 46: 893-911.

## 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.