SANTA CRUZ BIOTECHNOLOGY, INC.

GATA 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. Ko, L.J., et al. 1991. Murine and human T-lymphocyte GATA-3 factors mediate transcription through a *cis*-regulatory element within the human T cell receptor δ gene enhancer. Mol. Cell. Biol. 11: 2778-2784.

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.

PRODUCT

GATA CONSENSUS OLIGONUCLEOTIDE: sc-2531

- binding site for GATA-1, GATA-2, GATA-3 (3), GATA-4, GATA-5 and GATA-6

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5′– CAC  TTG  ATA  ACA  GAA  AGT  GAT  AAC  TCT –3′
3′– GTG  AAC  TAT  TGT  CTT  TCA  CTA  TTG  AGA –5′
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GATA MUTANT OLIGONUCLEOTIDE: sc-2532

- identical to sc-2531 with the exception of "GA"→"CT" substitutions in the DNA binding region (3)

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5′– CAC  TTG  ATA  ACA  GAA  AGT  CTT  AAC  TCT –3′
3′– GTG  AAC  TAT  TGT  CTT  TCA  GAA  TTG  AGA –5′
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SELECT PRODUCT CITATIONS

1. Peng, H.B., et al. 1995. Induction and stabilization of IκB-α by nitric oxide mediates inhibition of NFκB. J. Biol. Chem. 270: 14214-14219.
2. Ratziu, V., et al. 1998. Zf9, a Krüppel-like transcription factor up-regulated *in vivo* during early hepatic fibrosis. Proc. Natl. Acad. Sci. USA 95: 9500-9505.
3. Lauth, M., et al. 2000. Elevated perfusion pressure upregulates endothelin-1 and endothelin B receptor expression in the rabbit carotid artery. Hypertension 35: 648-654.
4. Finotto, S., et al. 2001. Treatment of allergic airway inflammation and hyperresponsiveness by antisense-induced local blockade of GATA-3 expression. J. Exp. Med. 193: 1247-1260.
5. Neurath, M.F., et al. 2002. The transcription factor T-bet regulates mucosal T cell activation in experimental colitis and Crohn's disease. J. Exp. Med. 195: 1129-1143.
6. Suzuki, Y.J., et al. 2003. Activation of GATA-4 by Serotonin in pulmonary artery smooth muscle cells. J. Biol. Chem. 278: 17525-17531.
7. Oesterreicher, T.J., et al. 2004. Rapid induction of GATA transcription factors in developing mouse intestine following glucocorticoid administration. Am. J. Physiol. Gastrointest. Liver Physiol. 286: G947-G953.
8. Yao, X., et al. 2009. Role of Stat3 and GATA-1 interactions in γ-globin gene expression. Exp. Hematol. 37: 889-900.
9. Jonckheere, N., et al. 2011. The mouse Muc5b mucin gene is transcriptionally regulated by thyroid transcription factor-1 (TTF-1) and GATA-6 transcription factors. FEBS J. 278: 282-294.

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.

RESEARCH USE

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