SANTA CRUZ BIOTECHNOLOGY, INC.

c-Myb 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. Guehmann, S., et al. 1992. Reduction of a conserved Cys is essential for Myb DNA-binding. Nucleic Acids Res. 20: 2279-2286.

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.

PROTOCOLS

See our web site at www.scbt.com for detailed protocols and support products.

PRODUCT

c-Myb CONSENSUS OLIGONUCLEOTIDE: sc-2583

- binding site for c-Myb (3)

```
5′– TAC  AGG   CAT  AAC  GGT  TCC  GTA  GTG  A – 3′
3′– ATG  TCC   GTA  TTG  CCA  AGG  CAT  CAC  T – 5′
```

c-Myb MUTANT OLIGONUCLEOTIDE: sc-2584

- identical to sc-2583 with the exception of an "A"→"T" substitution in the c-Myb binding motif (3)

```
5′– TAC  AGG   CAT  ATC  GGT  TCC  GTA  GTG  A – 3′
3′– ATG  TCC   GTA  TAG  CCA  AGG  CAT  CAC  T – 5′
```

SELECT PRODUCT CITATIONS

1. Tomita, A., et al. 2000. c-Myb acetylation at the carboxyl-terminal conserved domain by transcriptional co-activator p300. Oncogene 19: 444-451.
2. Kolonics, A., et al. 2001. Activation of Raf/ERK1/2 MAP kinase pathway is involved in GM-CSF-induced proliferation and survival but not in erythropoietin-induced differentiation of TF-1 cells. Cell. Signal. 13: 743-754.
3. Reizis, B., et al. 2001. The upstream enhancer is necessary and sufficient for the expression of the pre-T cell receptor α gene in immature T lymphocytes. J. Exp. Med. 194: 979-990.
4. Baek, W.K., et al. 2002. Molecular cloning and characterization of the human budding uninhibited by benomyl (BUB3) promoter. Gene 295: 117-123.
5. Green, R.C., et al. 2003. Germline hMLH1 promoter mutation in a Newfoundland HNPCC kindred. Clin. Genet. 64: 220-227.
6. Geng, C.D., et al. 2008. A conserved molecular mechanism is responsible for the auto-up-regulation of glucocorticoid receptor gene promoters. Mol. Endocrinol. 22: 2624-2642.
7. Schultz, J., et al. 2009. The functional -443T/C osteopontin promoter polymorphism influences osteopontin gene expression in melanoma cells via binding of c-Myb transcription factor. Mol. Carcinog. 48: 14-23.
8. Sade, H., et al. 2009. Transcriptional control of occludin expression in vascular endothelia: regulation by Sp3 and YY1. Biochim. Biophys. Acta 1789: 175-184.

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.