SANTA CRUZ BIOTECHNOLOGY, INC.

MEF-1 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. Nuceic 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. Neuhold, L.A., et al. 1993. HLH forced dimers: tethering MyoD to E47 generates a dominant positive myogenic factor insulated from negative regulation by Id. Cell 74: 1033-1042.

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

MEF-1 CONSENSUS OLIGONUCLEOTIDE: sc-2519

- binding site for MEF-1 specific transcription factors such as MyoD and myogenin (3)

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5'- GAT  CCC  CCC  AAC  ACC  TGC  TGC  CTG  A -3'
3'- CTA  GGG  GGG  TTG  TGG  ACG  ACG  GAC  T -5'
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MEF-1 MUTANT OLIGONUCLEOTIDE: sc-2520

- identical to sc-2519 with the exception of a six base pair substitution in the MEF-1 DNA binding motif (3)

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5'- GAT  CCC  CCC  AAC  ACG  GTA  ACC  CTG  A -3'
3'- CTA  GGG  GGG  TTG  TGC  CAT  TGG  GAC  T -5'
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SELECT PRODUCT CITATIONS

1. Nakayama, M., et al. 1996. Common core sequences are found in skeletal muscle slow and fast fiber type specific regulatory elements. Mol. Cell. Biol. 16: 2408-2417.
2. Takahashi, T., et al. 1997. A minimal murine Msx-1 gene promoter. Organization of its *cis*-regulatory motifs and their role in transcriptional activation in cells in culture and in transgenic mice. J. Biol. Chem. 272: 22667-22678.
3. Datta, B., et al. 1998. Increase in p202 expression during skeletal muscle differentiation: inhibition of MyoD protein expression and activity by p202. Mol. Cell. Biol. 18: 1074-1083.
4. Liu, C.J., et al. 2002. The MyoD-inducible p204 protein overcomes the inhibition of myoblast differentiation by Id proteins. Mol. Cell. Biol. 22: 2893-2905.

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.

PROTOCOLS

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