SANTA CRUZ BIOTECHNOLOGY, INC.

CDP 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. Aufiero, B., et al. 1994. Sequence-specific DNA binding of individual cut repeats of the human CCAAT displacement/cut homeodomain protein. Proc. Natl. Acad. Sci. USA 91: 7757-7761.

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.

RESEARCH USE

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

PRODUCT

CDP CONSENSUS OLIGONUCLEOTIDE: sc-2593

- binding site for CDP transcription factor (3)

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5′– ACC  CAA  TGA  TTA  TTA  GCC  AAT  TTC  TGA – 3′
3′– TGG  GTT  ACT  AAT  AAT  CGG  TTA  AAG  ACT – 5′
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CDP MUTANT OLIGONUCLEOTIDE: sc-2594

- identical to sc-2593 with the exception of "ATTA"→"GCCG" substitution in the CDP binding site (3)

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5′– ACC  CAA  TGG  CCG  TTA  GCC  AAT  TTC  TGA – 3′
3′– TGG  GTT  ACC  GGC  AAT  CGG  TTA  AAG  ACT – 5′
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SELECT PRODUCT CITATIONS

1. Michl, P., et al. 2006. CUTL1 is phosphorylated by protein kinase A, modulating its effects on cell proliferation and motility. J. Biol. Chem. 281: 15138-15144.
2. Rodríguez-Rodero, S., et al. 2007. Transcriptional regulation of MICA and MICB: a novel polymorphism in MICB promoter alters transcriptional regulation by Sp1. Eur. J. Immunol. 37: 1938-1953.
3. Ikeda, T., et al. 2016. Transforming growth factor-β-induced CUX1 isoforms are associated with fibrosis in systemic sclerosis lung fibroblasts. Biochem. Biophys. Rep. 7: 246-252.

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.

PROTOCOLS

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