SANTA CRUZ BIOTECHNOLOGY, INC.

HNF-4 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. Fraser, J.D., et al. 1998. DNA binding and transcription activation specificity of hepatocyte nuclear factor 4. Nucleic Acids Res. 26: 2702-2707.

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

HNF-4 CONSENSUS OLIGONUCLEOTIDE: sc-2599

- binding site for HNF-4 transcription factor (3)

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5′— CTC  AGC  TTG  TAC  TTT  GGT  ACA  ACT  A — 3′
3′— GAG  TCG  AAC  ATG  AAA  CCA  TGT  TGA  T — 5′
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HNF-4 MUTANT OLIGONUCLEOTIDE: sc-2600

- identical to sc-2599 with the exception of "G"→"C" and "T"→"A" substitutions in the HNF-4 binding motif (3)

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5′— CTC  AGC  TTC  TAC  TTA  GGT  ACA  ACT  A — 3′
3′— GAG  TCG  AAG  ATG  AAT  CCA  TGT  TGA  T — 5′
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SELECT PRODUCT CITATIONS

1. Stepanian, S.V., et al. 2003. Characterization of the human glycerol kinase promoter: identification of a functional HNF-4α binding site and evidence for transcriptional activation. Mol. Genet. Metab. 80: 412-418.
2. Cheng, P.Y., et al. 2003. Rapid transcriptional suppression of rat cytochrome P450 genes by endotoxin treatment and its inhibition by curcumin. J. Pharmacol. Exp. Ther. 307: 1205-1212.
3. Gupta, R.K., et al. 2005. The MODY1 gene HNF-4α regulates selected genes involved in Insulin secretion. J. Clin. Invest. 115: 1006-1015.
4. Sanguino, E., et al. 2005. Atorvastatin reverses age-related reduction in rat hepatic PPARα and HNF-4. Br. J. Pharmacol. 145: 853-861.

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.