NRSE Gel Shift 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.

GEL SHIFT ASSAYS

For gel shift analysis, nuclear extracts are prepared by the method of Dignam *et al.* (1). Oligonucleotides are ^{32}P-labeled with [γ^{32}P] ATP, using polynucleotide kinase (50,000 cpm/ng). Binding reaction mixtures (20λ) are incubated at room temperature for 20 minutes and contain 0.5 ng DNA probe and 3 to 10 µg nuclear extract in 10 mM Tris (pH 7.5) buffer with 50 mM NaCl, 1 mM dithiothreitol, 1 mM EDTA, 5% glycerol and 1 µg of poly (dI-dC) to inhibit non-specific binding of the labeled probe to nuclear extract proteins (2). DNA-protein complexes are resolved by electrophoresis through 4% poly-acrylamide gels containing 50 mM Tris, 0.38 M glycine and 2 mM EDTA. The gels are subsequently dried and autoradiographed with intensifying screens at -70° C.

GEL SUPERSHIFT ASSAYS

Gel supershift assays are performed as described above with the exception that subsequent to incubation of oligonucleotide probes with nuclear extracts, 1.0–2.0 µl of TransCruz gel supershift antibody (200 µg/ml) is added to the reaction mixture and incubated for 15–45 minutes at room temperature.

STORAGE

Store at -20° C; stable for one year from the date of shipment.

NRSE Gel Shift Oligonucleotides

sc-2631

• consensus binding site for NSRE; suitable for use with NSRF and CoREST (3)

5' - TTC AGC ACC ACG GAC AGC GCC - 3'
3' - AAG TCG TGG TGC CTG TCG CGG - 5'

NRSE Gel Shift Mutant Oligonucleotide

sc-2632

• identical to sc-2631 with the exception of a "CAGC" → "TCGT" and "GGAC" → "TCGT" substitution in the NSRE binding motif

5' - TTT CGT ACC ACT CGT AGC GCC - 3'
3' - AAA CGA TGG TGA CGA TCG CGG - 5'

BACKGROUND REFERENCES

1. Dignam, J.D., Lebovitz, R.M., and Roeder, R.G. 1983. Accurate transcription initiation by RNA polymerase II in a soluble extract from isolated mammalian nuclei. Nucl. Acids Res. 11: 1475-1489.

2. Murre, C., Voronova, A., and Baltimore, D. 1991. B-cell- and myocyte-specific E2-box-binding factors contain E12/E47-like subunits. Mol. Cell. Biol. 11: 1156-1160.

3. Schoenherr, C.J., Paquette, A.J., and Anderson, D.J. 1996. Identification of potential target genes for the neuron-restrictive silencer factor. Proc. Natl. Acad. Sci. USA 93: 9881-9886.

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