SANTA CRUZ BIOTECHNOLOGY, INC.

E2F-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. Nucl. 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. Lees, E., et al. 1992. Cyclin E/Cdk2 and cyclin A/Cdk2 kinases associate with p107 and E2F in a temporally distinct manner. Genes Dev. 6: 1874-1885.

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

E2F-1 CONSENSUS OLIGONUCLEOTIDE: sc-2507

- binding site for E2F-1 transcription factor (3)

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5′— ATT  TAA  GTT  TCG  CGC  CCT  TTC  TCA  A —3′
3′— TAA  ATT  CAA  AGC  GCG  GGA  AAG  AGT  T —5′
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E2F-1 MUTANT OLIGONUCLEOTIDE: sc-2508

- identical to sc-2507 with the exception of a "CG"→"AT"substitution in the E2F-1 binding motif (3)

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5′— ATT  TAA  GTT  TCG  ATC  CCT  TTC  TCA  A —3′
3′— TAA  ATT  CAA  AGC  TAG  GGA  AAG  AGT  T —5′
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SELECT PRODUCT CITATIONS

1. Rampalli, A.M., et al. 1998. pRB and p107 regulate E2F activity during lens fiber cell differentiation. Oncogene 16: 399-408.
2. Faenza, I., et al. 2000. A role for nuclear phospholipase Cβ 1 in cell cycle control. J. Biol. Chem. 275: 30520-30524.
3. Halaban, R., et al. 2000. Deregulated E2F transcriptional activity in autonomously growing melanoma cells. J. Exp. Med. 191: 1005-1016.
4. Advani, S.J., et al. 2000. E2F proteins are posttranslationally modified concomitantly with a reduction in nuclear binding activity in cells infected with herpes simplex virus 1. J. Virol. 74: 7842-7850.
5. Croxton, R., et al. 2002. Differences in DNA binding properties between E2F-1 and E2F-4 specify repression of the Mcl-1 promoter. Oncogene 21: 1563-1570.
6. Ma, Y., et al. 2003. Regulation of the cyclin D3 promoter by E2F-1. J. Biol. Chem. 278: 16770-16776.
7. Keenan, S.M., et al. 2004. Expression of cyclin E renders cyclin D-Cdk4 dispensable for inactivation of the retinoblastoma tumor suppressor protein, activation of E2F and G_1-S phase progression. J. Biol. Chem. 279: 5387-5396.
8. Sdek, P. 2004. The central acidic domain of MDM2 is critical in inhibition of retinoblastoma-mediated suppression of E2F and cell growth. J. Biol. Chem. 279: 53317-53322.
9. Sanz-González, S.M., et al. 2004. Role of E2F and ERK1/2 in STI571-mediated smooth muscle cell growth arrest and cyclin A transcriptional repression. Biochem. Biophys. Res. Commun. 317: 972-979.

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.