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Ets-1/PEA3 Consensus and Mutant Oligonucleotides

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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. Fisher, R.J., et al. 1992. Human Ets-1 oncoprotein. Purification, isoforms, SH modification and DNA sequence-specific binding. J. Biol. Chem. 267: 17957-17965.

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

Ets-1/PEA3 CONSENSUS OLIGONUCLEOTIDE: sc-2555

- binding site for Ets-1 and PEA3 (3)

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5' - GAT  CTC  GAG  CAG  GAA  GTT  CGA - 3'
3' - CTA  GAG  CTC  GTC  CTT  CAA  GCT - 5'
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Ets-1/PEA3 MUTANT OLIGONUCLEOTIDE: sc-2556

- identical to sc-2555 with the exception of a "G"→"A" substitution in the Ets-1/PEA3 binding motifs (3)

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5' - GAT  CTC  GAG  CAA  GAA  GTT  CGA - 3'
3' - CTA  GAG  CTC  GTT  CTT  CAA  GCT - 5'
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SELECT PRODUCT CITATIONS

1. Hoare, S., et al. 1999. Identification of a GABP α/β binding site involved in the induction of oxytocin receptor gene expression in human breast cells, potentiation by c-Fos/c-Jun. Endocrinology 140: 2268-2279.
2. Lauth, M., et al. 2000. Elevated perfusion pressure upregulates endothelin-1 and endothelin B receptor expression in the rabbit carotid artery. Hypertension 35: 648-654.
3. de Nigris, F., et al. 2001. Induction of ETS-1 and ETS-2 transcription factors is required for thyroid cell transformation. Cancer Res. 61: 2267-2275.
4. Furihata, K. and Kunicki, T.J. 2002. Characterization of human glycoprotein VI gene 5' regulatory and promoter regions. Arterioscler. Thromb. Vasc. Biol. 22: 1733-1739.
5. Liu, M., et al. 2003. Gene transcription of fgl2 in endothelial cells is controlled by Ets-1 and Oct-1 and requires the presence of both Sp1 and Sp3. Eur. J. Biochem. 270: 2274-2286.
6. Sevinsky, J.R., et al. 2004. Extracellular signal-regulated kinase induces the megakaryocyte GPIIb/CD41 gene through MafB/Kreisler. Mol. Cell. Biol. 24: 4534-4545.
7. Rouget, R., et al. 2005. Characterization of the survival motor neuron (SMN) promoter provides evidence for complex combinatorial regulation in undifferentiated and differentiated P19 cells. Biochem. J. 385: 433-443.
8. Zhang, Q.X., et al. 2006. Blockade of the translocation and activation of mitogen-activated protein kinase kinase 4 (MKK4) signaling attenuates neuronal damage during later ischemia-reperfusion. J. Neurochem. 98: 170-179.
9. Adams, J.P., et al. 2009. NMDA receptor-independent control of transcription factors and gene expression. Neuroreport 20: 1429-1433.
10. Watanabe, M., et al. 2012. Ets-1 activates overexpression of JunB and CD30 in Hodgkin's lymphoma and anaplastic large-cell lymphoma. Am. J. Pathol. 180: 831-838.

STORAGE

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

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