SANTA CRUZ BIOTECHNOLOGY, INC.

CREB 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. Roesler, W.J., et al. 1988. Cyclic AMP and the induction of eukaryotic gene transcription. J. Biol. Chem. 263: 9063-9066.
3. 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.

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

CREB CONSENSUS OLIGONUCLEOTIDE: sc-2504

- binding site for cAMP response element (CRE) binding proteins of the CREB/ATF family (3)

5' – AGA	GAT	TGC	CTG	ACG	TCA	GAG	AGC	TAG – 3'
3' – TCT	CTA	ACG	GAC	TGC	AGT	CTC	TCG	ATC – 5'

CREB MUTANT OLIGONUCLEOTIDE: sc-2517

- identical to sc-2504 with the exception of an "AC"→"TG"substitution in the CRE binding motif (3)

5' – AGA	GAT	TGC	CTG	TGG	TCA	GAG	AGC	TAG – 3'
3' – TCT	CTA	ACG	GAC	ACC	AGT	CTC	TCG	ATC – 5'

SELECT PRODUCT CITATIONS

1. Zhang, D.H., et al. 1997. Transcription factor GATA-3 is differentially expressed in murine Th1 and Th2 cells and controls Th2-specific expression of the interleukin-5 gene. J. Biol. Chem. 272: 21597-21603.
2. Castro-Caldas, M., et al. 2003. Dexamethasone-induced and estradiol-induced CREB activation and annexin 1 expression in CCRF-CEM lymphoblastic cells: evidence for the involvement of cAMP and p38 MAPK. Mediators Inflamm. 12: 329-337.
3. Dai, K.Z., et al. 2004. Transcriptional activation of the SH2D2A gene is dependent on a cyclic adenosine 5'-monophosphate-responsive element in the proximal SH2D2A promoter. J. Immunol. 172: 6144-6151.
4. White, P.C., et al. 2006. Regulation of cyclin D2 and the cyclin D2 promoter by protein kinase A and CREB in lymphocytes. Oncogene 25: 2170-2180.
5. Hay, C.W., et al. 2007. ATF-2 stimulates the human Insulin promoter through the conserved CRE2 sequence. Biochim. Biophys. Acta 1769: 79-91.
6. Aggarwal, S., et al. 2008. Growth suppression of lung cancer cells by targeting cyclic AMP response element-binding protein. Cancer Res. 68: 981-988.
7. Slonchak, A.M., et al. 2009. Crosstalk between transcription factors in regulation of the human glutathione S-transferase P1 gene expression in Me45 melanoma cells. Biopolym. Cell 25: 210-217.
8. Juvekar, A., et al. 2011. Bortezomib induces nuclear translocation of IκBα resulting in gene-specific suppression of NFκB-dependent transcription and induction of apoptosis in CTCL. Mol. Cancer Res. 9: 183-194.

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.