SANTA CRUZ BIOTECHNOLOGY, INC.

GAS/ISRE 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. Decker, T., et al. 1991. Cytoplasmic activation of GAF, an IFN-γ regulated DNA binding factor. EMBO J. 10: 927-932.

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

GAS/ISRE CONSENSUS OLIGONUCLEOTIDE: sc-2537

- binding site for interferon-γ activation factor (GAF) (3)

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5′— AAG  TAC  TTT  CAG  TTT  CAT  ATT  ACT  CTA — 3′
3′— TTC  ATG  AAA  GTC  AAA  GTA  TAA  TGA  GAT — 5′
                      ISRE                GAS
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GAS/ISRE MUTANT OLIGONUCLEOTIDE: sc-2538

- identical to sc-2537 with the exception of "TTCA"→"GGTC" substitution in the overlapping region of the GAS and ISRE DNA binding elements (3)

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5′— AAG  TAC  TTT  CAG  TGG  TCT  ATT  ACT  CTA — 3′
3′— TTC  ATG  AAA  GTC  ACC  AGA  TAA  TGA  GAT — 5′
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SELECT PRODUCT CITATIONS

1. Vaughan, P.S., et al. 1995. Activation of a cell-cycle-regulated histone gene by the oncogenic transcription factor IRF-2. Nature 377: 362-365.
2. Bourcier, T., et al. 1997. The nuclear factor κB signaling pathway participates in dysregulation of vascular smooth muscle cells *in vitro* and in human atherosclerosis. J. Biol. Chem. 272: 15817-15824.
3. Dumler, I., et al. 1998. The JAK/Stat pathway and urokinase receptor signaling in human aortic vascular smooth muscle cells. J. Biol. Chem. 273: 315-321.
4. Lukiw, W.J., et al. 1998. Budesonide epimer R or Dexamethasone selectively inhibit platelet-activating factor-induced or interleukin 1β-induced DNA binding activity of *cis*-acting transcription factors and cyclooxygenase-2 gene expression in human epidermal keratinocytes. Proc. Natl. Acad. Sci. USA 95: 3914-3919.
5. Kim, H.Y., et al. 2003. Curcumin suppresses Janus kinase-Stat inflammatory signaling through activation of Src homology 2 domain-containing tyrosine phosphatase 2 in brain microglia. J. Immunol. 171: 6072-6079.
6. Adams, J., et al. 2005. 13-*cis* retinoic acid inhibits development and progression of chronic allograft nephropathy. Am. J. Pathol. 167: 285-298.
7. Chen, Y., et al. 2007. Proteomic identification of proteins associated with the osmoregulatory transcription factor TonEBP/OREBP: functional effects of Hsp90 and PARP-1. Am. J. Physiol. Renal Physiol. 292: F981-F992.

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.