SANTA CRUZ BIOTECHNOLOGY, INC.

IRF-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. 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. Harada, H., et al. 1994. Structure and regulation of the human interferon regulatory factor 1 (IRF-1) and IRF-2 genes: implications for a gene network in the interferon system. Mol. Cell. Biol. 14: 1500-1509.

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

IRF-1 CONSENSUS OLIGONUCLEOTIDE: sc-2575

- binding site for IRF-1 (3)

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5'— GGA  AGC  GAA  AAT  GAA  ATT  GAC  T — 3'
3'— CCT  TCG  CTT  TTA  CTT  TAA  CTG  A — 5'
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IRF-1 MUTANT OLIGONUCLEOTIDE: sc-2576

- identical to sc-2575 with the exception of two "AA"→"GG" substitutions in the IRF-1 binding motif (3)

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5'— GGA  AGC  GAG  GAT  GAG  GTT  GAC  T — 3'
3'— CCT  TCG  CTC  CTA  CTC  CAA  CTG  A — 5'
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SELECT PRODUCT CITATIONS

1. Chatterjee-Kishore, M., et al. 1998. Different requirements for signal transducer and activator of transcription 1α and interferon regulatory factor 1 in the regulation of low molecular mass polypeptide 2 and transporter associated with antigen processing 1 gene expression. J. Biol. Chem. 273: 16177-16183.
2. Fessele, S., et al. 2001. Molecular and in silico characterization of a promoter module and C/EBP element that mediate LPS-induced RANTES/CCL5 expression in monocytic cells. FASEB J. 15: 577-579.
3. Wagner, A.H., et al. 2002. Cytokine-inducible CD40 expression in human endothelial cells is mediated by interferon regulatory factor-1. Blood 99: 520-525.
4. Dekoninck, A., et al. 2003. Identification and characterization of a PU.1/Spi-B binding site in the bovine leukemia virus long terminal repeat. Oncogene 22: 2882-2896.
5. Naschberger, E., et al. 2004. Nuclear factor-κB motif and interferon-α-stimulated response element co-operate in the activation of guanylate-binding protein-1 expression by inflammatory cytokines in endothelial cells. Biochem. J. 379: 409-420.
6. Guo, Z., et al. 2005. A distal regulatory region is required for constitutive and IFN-β-induced expression of murine TLR9 gene. J. Immunol. 175: 7407-7418.
7. Luo, X.M. and Ross, A.C. 2006. Retinoic acid exerts dual regulatory actions on the expression and nuclear localization of interferon regulatory factor-1. Exp. Biol. Med. 231: 619-631.
8. Byun, S.J., et al. 2007. IFN-γ upregulates expression of the mouse complement C1rA gene in keratinocytes via IFN-regulatory factor-1. J. Invest. Dermatol. 127: 1187-1196.
9. Rodríguez, T., et al. 2007. Distinct mechanisms of loss of IFN-γ mediated HLA class I inducibility in two melanoma cell lines. BMC Cancer 7: 34.

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.