SANTA CRUZ BIOTECHNOLOGY, INC.

SRE 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. Attar, R.M., et al. 1992. Expression cloning of a novel zinc finger protein that binds to the c-Fos serum response element. Mol. Cell. Biol. 12: 2432-2443.

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

SRE CONSENSUS OLIGONUCLEOTIDE: sc-2523

- binding site for SRF and related transcription factors (3)

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5′– GGA  TGT  CCA  TAT  TAG  GAC  ATC  T – 3′
3′– CCT  ACA  GGT  ATA  ATC  CTG  TAG  A – 5′
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SRE MUTANT OLIGONUCLEOTIDE: sc-2524

- identical to sc-2523 with the exception of a "GG"→"TT" substitution in the DNA binding region (3)

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5′– GGA  TGT  CCA  TAT  TAT  TAC  ATC  T – 3′
3′– CCT  ACA  GGT  ATA  ATA  ATG  TAG  A – 5′
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SELECT PRODUCT CITATIONS

1. Cui, M.Z., et al. 1999. Native and oxidized low density lipoprotein induction of tissue factor gene expression in smooth muscle cells is mediated by both Egr-1 and Sp1. J. Biol. Chem. 274: 32795-32802.
2. Ding, W., et al. 1999. Transformation blocks differentiation-induced inhibition of serum response factor interactions with serum response elements. Cancer Res. 59: 3795-3802.
3. Lin, H., et al. 2003. Rapid electrical and delayed molecular signals regulate the serum response element after nerve injury: convergence of injury and learning signals. J. Neurobiol. 57: 204-220.
4. Cui, M.Z., et al. 2006. Lysophosphatidic acid induces early growth response gene 1 expression in vascular smooth muscle cells: CRE and SRE mediate the transcription. Arterioscler. Thromb. Vasc. Biol. 26: 1029-1035.
5. Herrmann, J., et al. 2007. TGF-β up-regulates serum response factor in activated hepatic stellate cells. Biochim. Biophys. Acta 1772: 1250-1257.
6. Madonna, R., et al. 2014. Co-activation of nuclear factor-κB and Myocardin/serum response factor conveys the hypertrophy signal of high Insulin levels in cardiac myoblasts. J. Biol. Chem. 289: 19585-19598.
7. Shankar, E., et al. 2016. Signaling network controlling androgenic repression of c-Fos in prostate adenocarcinoma cells. J. Biol. Chem. 291: 5512-5526.

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.

PROTOCOLS

See our web site at www.scbt.com for detailed protocols and support products.