SANTA CRUZ BIOTECHNOLOGY, INC.

USF-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. Hoffman, P.W., et al. 1995. DNA binding and regulatory effects of transcription factors Sp1 and USF at the rat Amyloid precursor protein gene promoter. Nucleic Acids Res. 23: 2229-2235.

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

USF-1 CONSENSUS OLIGONUCLEOTIDE: sc-2557

- binding site for USF-1 (3)

5' – CAC CCG GTC ACG TGG CCT ACA CC – 3'
3' – GTG GGC CAG TGC ACC GGA TGT GG – 5'

USF-1 MUTANT OLIGONUCLEOTIDE: sc-2558

- identical to sc-2557 with the exception of a "CG"→"AT" substitution in the USF-1 binding motif (3)

5' – CAC CCG GTC A**AT** TGG CCT ACA CC – 3'
3' – GTG GGC CAG TTA ACC GGA TGT GG – 5'

SELECT PRODUCT CITATIONS

1. Malyankar, U.M., et al. 1999. Upstream stimulatory factor 1 regulates osteopontin expression in smooth muscle cells. Exp. Cell Res. 250: 535-547.
2. Nishikawa, N.S., et al. 2000. Cloning and characterization of the 5'-upstream sequence governing the cell cycle-dependent transcription of mouse DNA polymerase α 68 kDa subunit gene. Nucleic Acids Res. 28: 1525-1534.
3. Ge, Y., et al. 2001. Transcriptional regulation of the human cystathionine β-synthase -1b basal promoter: synergistic transactivation by transcription factors NF-Y and Sp1/Sp3. Biochem. J. 357: 97-105.
4. Saur, D., et al. 2002. Complex regulation of human neuronal nitric-oxide synthase exon 1c gene transcription. Essential role of Sp and ZNF family members of transcription factors. J. Biol. Chem. 277: 25798-25814.
5. Lee, S.S., et al. 2003. Cloning and characterization of the rat Hsf2 promoter: a critical role of proximal E-box element and USF protein in Hsf2 regulation in different compartments of the brain. Biochim. Biophys. Acta 1625: 52-63.
6. Narvaez, M.J., et al. 2005. Characterization of adjacent E-box and nuclear factor 1-like DNA binding sequence in the human CYP1A2 promoter. J. Biochem. Mol. Toxicol. 19: 78-86.
7. Bélanger, A.S., et al. 2010. Regulation of UGT1A1 and HNF1 transcription factor gene expression by DNA methylation in colon cancer cells. BMC Mol. Biol. 11: 9.
8. Reichenbach, G., et al. 2013. PPARα agonist Wy14643 suppresses cathepsin B in human endothelial cells via transcriptional, post-transcriptional and post-translational mechanisms. Angiogenesis 16: 223-233.
9. Maples, J.M., et al. 2015. Differential epigenetic and transcriptional response of the skeletal muscle carnitine palmitoyltransferase 1B (CPT1B) gene to lipid exposure with obesity. Am. J. Physiol. Endocrinol. Metab. 309: E345-E356.

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.