SANTA CRUZ BIOTECHNOLOGY, INC.

ER 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. Klinge, C.M., et al. 1992. Cooperative estrogen receptor interaction with consensus or variant estrogen response elements *in vitro*. Cancer Res. 52: 1073-1081.

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

ER CONSENSUS OLIGONUCLEOTIDE: sc-2585

- ERE consensus binding site for ER transcription factor (3)

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5′– GGA  TCT  AGG  TCA   CTG  TGA  CCC  CGG  ATC – 3′
3′– CCT  AGA  TCC  AGT   GAC  ACT  GGG  GCC  TAG – 5′
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ER MUTANT OLIGONUCLEOTIDE: sc-2586

- identical to sc-2585 with the exception of a "GT"→"TA" substitution in the ER binding site (3)

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5′– GGA  TCT  AGT  ACA   CTG  TGA  CCC  CGG  ATC – 3′
3′– CCT  AGA  TCA  TGT   GAC  ACT  GGG  GCC  TAG – 5′
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SELECT PRODUCT CITATIONS

1. LaVoie, H.A., et al. 2002. Cloning and characterization of porcine ovarian estrogen receptor β isoforms. Biol. Reprod. 66: 616-623.
2. Szabo, P.E., et al. 2004. Parent-of-origin-specific binding of nuclear hormone receptor complexes in the H19-Igf2 imprinting control region. Mol. Cell. Biol. 24: 4858-4868.
3. Sumi, D., et al. 2005. Sp1 transcription factor expression is regulated by estrogen-related receptor α1. Biochem. Biophys. Res. Commun. 328: 165-172.
4. Gururaj, A.E., et al. 2006. MTA1, a transcriptional activator of breast cancer amplified sequence 3. Proc. Natl. Acad. Sci. USA 103: 6670-6675.
5. Kundu, P., et al. 2007. Regulation of mouse Slo gene expression: multiple promoters, transcription start sites, and genomic action of estrogen. J. Biol. Chem. 282: 27478-27492.
6. Kundu, P., et al. 2008. Hormonal regulation of cardiac KCNE2 gene expression. Mol. Cell. Endocrinol. 292: 50-62.
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. Slonchak, A.M., et al. 2009. Transcription regulation in differential expression of the human GSTP1 gene in breast and choriocarcinoma cells. Ukr. Biokhim. Zh. 81: 48-58.
9. Jung, H.H., et al. 2010. Matrix metalloproteinase-1 expression can be upregulated through mitogen-activated protein kinase pathway under the influence of human epidermal growth factor receptor 2 synergized with estrogen receptor. Mol. Cancer Res. 8: 1037-1047.
10. Chun, J., et al. 2014. The induction of apoptosis by a newly synthesized diosgenyl saponin through the suppression of estrogen receptor-α in MCF-7 human breast cancer cells. Arch. Pharm. Res. 37: 1477-1486.

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.