SANTA CRUZ BIOTECHNOLOGY, INC.

RAR (DR-5) 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. Leid, M., et al. 1992. Purification, cloning and RXR identity of the HeLa cell factor with which RAR or TR heterodimerizes to bind target sequences efficiently. Cell 68: 377-395.

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

RAR (DR-5) CONSENSUS OLIGONUCLEOTIDE: sc-2559

- binding site for the retinoic acid receptor (3)

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5′– TCG AGG GTA GGG TTC ACC GAA AGT TCA CTC G –3′
3′– AGC TCC CAT CCC AAG TGG CTT TCA AGT GAG C –5′
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RAR (DR-5) MUTANT OLIGONUCLEOTIDE: sc-2560

- identical to sc-2559 with the exception of two "TT"→"AA" substitutions in the RAR binding motif (3)

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5′– TCG AGG GTA GGG AAC ACC GAA AGA ACA CTC G –3′
3′– AGC TCC CAT CCC TTG TGG CTT TCT TGT GAG C –5′
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SELECT PRODUCT CITATIONS

1. Shimizu, T. and Takeda, K. 2000. Induction of retinoic acid receptor-α by granulocyte macrophage colony-stimulating factor in human myeloid leukemia cell lines. Cancer Res. 60: 4544-4549.
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. Song, J., et al. 2004. Cyclophilin A is required for retinoic acid-induced neuronal differentiation in p19 cells. J. Biol. Chem. 279: 24414-24419.
4. Preston, I.R., et al. 2005. Retinoids and pulmonary hypertension. Circulation 111: 782-790.
5. Gonsky, R., et al. 2006. An IFNG SNP with an estrogen-like response element selectively enhances promoter expression in peripheral but not lamina propria T cells. Genes Immun. 7: 342-351.
6. Cohen, A.J., et al. 2006. Retinoids directly activate the collagen X promoter in prehypertrophic chondrocytes through a distal retinoic acid response element. J. Cell. Biochem. 99: 269-278.
7. 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.
8. 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.
9. Masuda, M., et al. 2010. Regulation of renal sodium-dependent phosphate co-transporter genes (Npt2a and Npt2c) by all-*trans*-retinoic acid and its receptors. Biochem. J. 429: 583-592.

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.