SANTA CRUZ BIOTECHNOLOGY, INC.

GR 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. Tanaka, H., et al. 1991. Identification and characterization of a *cis*-acting element that interferes with glucocorticoid-inducible activation of the mouse mammary tumor virus promoter. Proc. Natl. Acad. Sci. USA 88: 5393-5397.

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

GR CONSENSUS OLIGONUCLEOTIDE: sc-2545

- binding site for the glucocorticoid receptor (3)

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5′— GAC  CCT  AGA  GGA  TCT GTA  CAG GAT GTT  CTA GAT —3′
3′? CTG  GGA  TCT  CCT  AGA CAT  GTC CTA CAA  GAT CTA —5′
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GR MUTANT OLIGONUCLEOTIDE: sc-2546

- identical to sc-2545 with the exception of two "GT"→"CA" substitutions in the GR binding motif (3)

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5′— GAC  CCT  AGA  GGA  TCT CAA  CAG GAT CAT  CTA GAT —3′
3′? CTG  GGA  TCT  CCT  AGA GTT  GTC CTA GTA  GAT CTA —5′
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SELECT PRODUCT CITATIONS

1. Di Battista, J.A., et al. 1999. Enhancement of phosphorylation and transcriptional activity of the glucocorticoid receptor in human synovial fibroblasts by nimesulide, a preferential cyclooxygenase 2 inhibitor. Arthritis Rheum. 42: 157-166.
2. Pelletier, J.P., et al. 1999. Effect of nimesulide on glucocorticoid receptor activity in human synovial fibroblasts. Rheumatology 38: 11-13.
3. Kizaki, T., et al. 2000. Age-associated increase of basal corticosterone levels decreases ED2^{high}, NFκB^{high} activated macrophages. J. Leukoc. Biol. 68: 21-30.
4. Chen, Y., et al. 2006. Dexamethasone-mediated repression of MUC5AC gene expression in human lung epithelial cells. Am. J. Respir. Cell Mol. Biol. 34: 338-347.
5. Kook, I., et al. 2015. Bovine herpesvirus 1 productive infection and immediate early transcription unit 1 promoter are stimulated by the synthetic corticosteroid dexamethasone. Virology 484: 377-385.
6. Eisa, N.H., et al. 2019. The co-chaperone UNC45A is essential for the expression of mitotic kinase NEK7 and tumorigenesis. J. Biol. Chem. 294: 5246-5260.

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.

PROTOCOLS

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