

PR 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. *Nucl. Acids Res.* 11: 1475-1489.
2. Klein-Hitpass, L., et al. 1990. The progesterone receptor stimulates cell-free transcription by enhancing the formation of a stable preinitiation complex. *Cell* 60: 247-257.
3. 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.

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 [³²P]-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

PR CONSENSUS OLIGONUCLEOTIDE: sc-2543

- binding site for the progesterone receptor (3)

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5' - GAT CC T GTA CAG GAT GTT CTA GCT ACA - 3'
3' - CTA GGA CAT GTC CTA CAA GAT CGA TGT - 5'
  
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PR MUTANT OLIGONUCLEOTIDE: sc-2544

- identical to sc-2543 with the exception of two "GT" → "CA" substitutions in the PR binding motif (3)

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5' - GAT CC T CAA CAG GAT CAT CTA GCT ACA - 3'
3' - CTA GGA GTT GTC CTA GTA GAT CGA TGT - 5'
  
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SELECT PRODUCT CITATIONS

1. Labriola, L., et al. 2003. Heregulin induces transcriptional activation of the progesterone receptor by a mechanism that requires functional ErbB-2 and mitogen-activated protein kinase activation in breast cancer cells. *Mol. Cell. Biol.* 23: 1095-1111.
2. Romics, L., et al. 2004. Diverse regulation of NF-κB and peroxisome proliferator-activated receptors in murine nonalcoholic fatty liver. *Hepatology* 40: 376-385.
3. Katiyar, P., et al. 2009. Mechanism of BRCA1-mediated inhibition of progesterone receptor transcriptional activity. *Mol. Endocrinol.* 23: 1135-1146.

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.