SANTA CRUZ BIOTECHNOLOGY, INC.

AR 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. Roche, P.J., et al. 1992. A consensus DNA-binding site for the androgen receptor. Mol. Endocrinol. 6: 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

AR CONSENSUS OLIGONUCLEOTIDE: sc-2551

- binding site for the androgen receptor (3)

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5′–CTA  GAA  GTC  TGG  TAC  AGG  GTG  TTC  TTT  TTG  CA –3′
3′–GAT  CTT  CAG  ACC  ATG  TCC  CAC  AAG  AAA  AAC  GT –5′
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AR MUTANT OLIGONUCLEOTIDE: sc-2552

- identical to sc-2551 with the exception of two "GT"→"CA" substitutions in the AR binding motif (3)

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5′–CTA  GAA  GTC  TGC  AAC  AGG  GTC  ATC  TTT  TTG  CA –3′
3′–GAT  CTT  CAG  ACG  TTG  TCC  CAG  TAG  AAA  AAC  GT –5′
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SELECT PRODUCT CITATIONS

1. Kumar, S., et al. 1998. NT-3-mediated TrkC receptor activation promotes proliferation and cell survival of rodent progenitor oligodendrocyte cells *in vitro* and *in vivo*. J. Neurosci. Res. 54: 754-765.
2. Phan, D., et al. 2001. Androgen regulation of the cell-cell adhesion molecule-1 (CEACAM1) gene. Mol. Cell. Endocrinol. 184: 115-123.
3. Leman, E.S. and Getzenberg, R.H. 2003. Effects of 1,25-dihydroxyvitamin D_3 on the distribution of androgen and vitamin D receptors in human prostate neonatal epithelial cells. J. Cell. Biochem. 88: 609-622.
4. Park, S.Y., et al. 2007. Peroxiredoxin 1 interacts with androgen receptor and enhances its transactivation. Cancer Res. 67: 9294-9303.
5. Zhu, Y., et al. 2013. RhoGDIα downregulates androgen receptor signaling in prostate cancer cells. Prostate 73: 1614-1622.
6. Sánchez-González, C., et al. 2014. Walnut polyphenol metabolites, urolithins A and B, inhibit the expression of the prostate-specific antigen and the androgen receptor in prostate cancer cells. Food Funct. 5: 2922-2930.
7. Jeong, Y.H., et al. 2016. Lonchocarpine increases Nrf2/ARE-mediated antioxidant enzyme expression by modulating AMPK and MAPK signaling in brain astrocytes. Biomol. Ther. 24: 581-588.
8. Lee, Y.Y., et al. 2016. Anti-inflammatory and antioxidant mechanism of tangeretin in activated microglia. J. Neuroimmune Pharmacol. 11: 294-305.
9. Park, J.S., et al. 2016. β-Lapachone increases phase II antioxidant enzyme expression via NQO1-AMPK/PI3K-Nrf2/ARE signaling in rat primary astrocytes. Free Radic. Biol. Med. 97: 168-178.
10. Yu, X., et al. 2020. Structural insights of transcriptionally active, full-length androgen receptor coactivator complexes. Mol. Cell 79: 812-823.e4.
11. Yu, X., et al. 2022. Spatial definition of the human progesterone receptor-B transcriptional complex. iScience 25: 105321.

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.