SANTA CRUZ BIOTECHNOLOGY, INC.

VDR (DR-3) 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. Umesono, K., et al. 1991. Direct repeats as selective response elements for the thyroid hormone, retinoic acid and vitamin D_3 receptors. Cell 65: 1255-1266.

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

VDR (DR-3) CONSENSUS OLIGONUCLEOTIDE: sc-2539

- binding site for the Vitamin D receptor (3)

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5′— AGC  TTC  AGG  TCA  AGG  AGG  TCA  GAG  AGC  T — 3′
3′— TCG  AAG  TCC  AGT  TCC  TCC  AGT  CTC  TCG  A — 5′
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VDR (DR-3) MUTANT OLIGONUCLEOTIDE: sc-2540

- identical to sc-2539 with the exception of two "GT"→"AA" substitutions in the Vitamin D receptor binding motif (3)

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5′— AGC  TTC  AGAACA  AGG  AGAACA  GAG  AGC  T — 3′
3′— TCG  AAG  TCT TGT  TCC  TCT TGT  CTC  TCG  A — 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. Yeung, F., et al. 2002. Regulation of human osteocalcin promoter in hormone-independent human prostate cancer cells. J. Biol. Chem. 277: 2468-2476.
3. Qi, X., et al. 2002. The p38 and JNK pathways cooperate to transactivate vitamin D receptor via c-Jun/AP-1 and sensitize human breast cancer cells to vitamin D_3-induced growth inhibition. J. Biol. Chem. 277: 25884-25892.
4. Leman, E.S., et al. 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.
5. Zhou, Y., et al. 2009. Compound heterozygous mutations in the vitamin D receptor in a patient with hereditary 1,25-dihydroxyvitamin D-resistant rickets with alopecia. J. Bone Miner. Res. 24: 643-651.

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.