SANTA CRUZ BIOTECHNOLOGY, INC.

TR (DR-4) 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 D3 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.

PRODUCT

TR (DR-4) CONSENSUS OLIGONUCLEOTIDE: sc-2563

- binding site for the thyroid hormone receptor (3)

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5′– AGC  TTC  AGG  TCA  CAG  GAG  GTC  AGA  GAG  CT – 3′
3′– TCG  AAG  TCC  AGT  GTC  CTC  CAG  TCT  CTC  GA – 5′
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TR (DR-4) MUTANT OLIGONUCLEOTIDE: sc-2564

- identical to sc-2563 with the exception of two "GT"→"AA" substitutions in the TR binding motif (3)

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5′– AGC  TTC  AGA  ACA  CAG  GAG  AAC  AGA  GAG  CT – 3′
3′– TCG  AAG  TCT  TGT  GTC  CTC  TTG  TCT  CTC  GA – 5′
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SELECT PRODUCT CITATIONS

1. Thomas, W., et al. 2002. Thyroid hormone is a critical determinant for the regulation of the cochlear motor protein prestin. Proc. Natl. Acad. Sci. USA 99: 2901-2906.
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. Dobretsova, A., et al. 2004. Potentiation of myelin proteolipid protein (Plp) gene expression is mediated through AP-1-like binding sites. J. Neurochem. 90: 1500-1510.
4. García-G, C., et al. 2007. 3,5-Diiodothyronine *in vivo* maintains euthyroidal expression of type 2 iodothyronine deiodinase, growth hormone, and thyroid hormone receptor β1 in the killifish. Am. J. Physiol. Regul. Integr. Comp. Physiol. 293: R877-R883.
5. Macoritto, M., et al. 2008. Phosphorylation of the human retinoid X receptor α at serine 260 impairs coactivator(s) recruitment and induces hormone resistance to multiple ligands. J. Biol. Chem. 283: 4943-4956.
6. Lassová, L., et al. 2009. Thyroid hormone treatment of cultured chondrocytes mimics *in vivo* stimulation of collagen X mRNA by increasing BMP 4 expression. J. Cell. Physiol. 219: 595-605.
7. Kozai, M., et al. 2013. Thyroid hormones decrease plasma 1α,25-dihydroxyvitamin D levels through transcriptional repression of the renal 25-hydroxyvitamin D_3 1α-hydroxylase gene (CYP27B1). Endocrinology 154: 609-622.

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.