SANTA CRUZ BIOTECHNOLOGY, INC.

PPAR Consensus and Mutant Oligonucleotides

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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. Juge-Aubry, C., et al. 1997. DNA binding properties of peroxisome proliferator-activated receptor subtypes on various natural peroxisome proliferator response elements. J. Biol. Chem. 272: 25252-25259.

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

PPAR CONSENSUS OLIGONUCLEOTIDE: sc-2587

- binding site for PPARα, PPARβ, PPARγ transcription factors (3)

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5′– CAA  AAC  TAG  GTC  AA A  GGT  CA – 3′
3′– GTT  TTG  ATC  CAG  TTT  CCA  GT – 5′
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PPAR MUTANT OLIGONUCLEOTIDE: sc-2588

- identical to sc-2587 with the exception of two "GT"→"CA" substitutions in the PPAR/RXR binding motif (3)

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5′– CAA  AAC  TAG  CAC  AA A  GCA  CA – 3′
3′– GTT  TTG  ATC  GTG  TTT  CGT  GT – 5′
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SELECT PRODUCT CITATIONS

1. Cabrero, A., et al. 2003. Down-regulation of acyl-CoA oxidase gene expression and increased NFκB activity in etomoxir-induced cardiac hypertrophy. J. Lipid Res. 44: 388-398.
2. Eibl, G., et al. 2005. Growth stimulation of COX-2-negative pancreatic cancer by a selective COX-2 inhibitor. Cancer Res. 65: 982-990.
3. Subbarayan, V., et al. 2006. 15-lipoxygenase-2 gene regulation by its product 15-(S)-hydroxyeicosatetraenoic acid through a negative feedback mechanism that involves peroxisome proliferator-activated receptor γ. Oncogene 25: 6015-6025.
4. Ho, T.C., et al. 2007. PEDF induces p53-mediated apoptosis through PPAR γ signaling in human umbilical vein endothelial cells. Cardiovasc. Res. 76: 213-223.
5. Perez, A., et al. 2008. Peroxisome proliferator-activated receptor-γ in cystic fibrosis lung epithelium. Am. J. Physiol. Lung Cell. Mol. Physiol. 295: L303-L313.
6. Cho, H.Y., et al. 2010. Nrf2-regulated PPARγ expression is critical to protection against acute lung injury in mice. Am. J. Respir. Crit. Care Med. 182: 170-182.
7. Mahali, S.K., et al. 2012. β-D-glucoside protects against advanced glycation end products (AGEs)-mediated diabetic responses by suppressing ERK and inducing PPAR γ DNA binding. Biochem. Pharmacol. 84: 1681-1690.

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.