PPAR Consensus and Mutant Oligonucleotides

SANTA CRUZ® BIOTECHNOLOGY

The Power to Question

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.

RESEARCH USE

For research use only, not for use in diagnostic procedures.

PRODUCT

PPAR CONSENSUS OLIGONUCLEOTIDE: sc-2587

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

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5' – CAA  AAC  T AG  GTC  A AA  GGT  CA – 3'
3' – GTT  TTG  A TC  CAG  T TT  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  T AG  CAC  A AA  GCA  CA – 3'
3' – GTT  TTG  A TC  GTG  T TT  CGT  GT – 5'
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SELECT PRODUCT CITATIONS

1. Erl, W., et al. 2004. Cyclopentenone prostaglandins induce endothelial cell apoptosis independent of the peroxisome proliferator-activated receptor-γ. Eur. J. Immunol. 34: 241-250.

2. Han, K.H., et al. 2005. HMG-CoA reductase inhibition reduces monocyte CC chemokine receptor 2 expression and monocyte chemoattractant protein-1-mediated monocyte recruitment *in vivo*. Circulation 111: 1439-1447.

3. Relic, B., et al. 2006. Peroxisome proliferator-activated receptor-γ1 is dephosphorylated and degraded during BAY 11-7085-induced synovial fibroblast apoptosis. J. Biol. Chem. 281: 22507-22604.

4. Sanguino, E., et al. 2006. Ageing introduces a complex pattern of changes in several rat brain transcription factors depending on gender and anatomical localization. Exp. Gerontol. 41: 372-379.

5. Ho, T.C., et al. 2007. PEDF induces p53-mediated apoptosis through PPARγ signaling in human umbilical vein endothelial cells. Cardiovasc. Res. 76: 213-223.

6. Amoruso, A., et al. 2009. Enhanced peroxisome proliferator-activated receptor-γ expression in monocyte/macrophages from coronary artery disease patients and possible gender differences. J. Pharm. Exp. Ther. 331: 531-538.

7. Kim, H.B., et al. 2010. Lipin 1 represses NFATc4 transcriptional activity in adipocytes to inhibit secretion of inflammatory factors. Mol. Cell. Biol. 30: 3126-3139.

8. 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.

9. Zeng, H., et al. 2011. Anti-inflammatory properties of clovamide and Theobroma cacao phenolic extracts in human monocytes: evaluation of respiratory burst, cytokine release, NFκB activation, and PPARγ modulation. J. Agric. Food Chem. 59: 5342-5350.

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.