SANTA CRUZ BIOTECHNOLOGY, INC.

p53 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. Kastan, M.B., et al. 1992. A mammalian cell cycle checkpoint pathway utilizing p53 and GADD45 is defective in ataxia-telangiectasia. Cell 71: 587-597.

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

p53 CONSENSUS OLIGONUCLEOTIDE: sc-2579

- binding site for p53 (3)

5' – TAC AGA ACA TGT CTA AGC ATG CTG GGG ACT – 3'
3' – ATG TCT TGT ACA GAT TCG TAC GAC CCC TGA – 5'

p53 MUTANT OLIGONUCLEOTIDE: sc-2580

- identical to sc-2579 with the exception of a "CATG"→"TCGC" substitution in the p53 binding motif (3)

5' – TAC AGA ATC GCT CTA AGC ATG CTG GGG ACT – 3'
3' – ATG TCT TAG CGA GAT TCG TAC GAC CCC TGA – 5'

SELECT PRODUCT CITATIONS

1. Middeler, G., et al. 1997. The tumor suppressor p53 is subject to both nuclear import and export, and both are fast, energy-dependent and lectin-inhibited. Oncogene 14: 1407-1417.
2. Liu, G.Y., et al. 1998. Induction of apoptosis by thiuramdisulfides, the reactive metabolites of dithiocarbamates, through coordinative modulation of NFκB, c-fos/c-jun, and p53 proteins. Mol. Carcinog. 22: 235-246.
3. Jimenez, G.S., et al. 1999. DNA-dependent protein kinase is not required for the p53-dependent response to DNA damage. Nature 400: 81-83.
4. Luo, J., et al. 2001. Knock-in mice with a chimeric human/murine p53 gene develop normally and show wildtype p53 responses to DNA damaging agents: a new biomedical research tool. Oncogene 20: 320-328.
5. Saur, D., et al. 2002. Complex regulation of human neuronal nitric-oxide synthase exon 1c gene transcription. Essential role of Sp and ZNF family members of transcription factors. J. Biol. Chem. 277: 25798-25814.
6. Ying, H., et al. 2005. DNA-binding and transactivation activities are essential for TA* p63 protein degradation. Mol. Cell. Biol. 25: 6154-6164.
7. Tang, F., et al. 2006. Arsenite inhibits p53 phosphorylation, DNA binding activity, and p53 target gene p21 expression in mouse epidermal JB6 cells. Mol. Carcinog. 45: 861-870.
8. Venkatesan, B., et al. 2010. WNT1-inducible signaling pathway protein-1 activates diverse cell survival pathways and blocks doxorubicin-induced cardiomyocyte death. Cell. Signal. 22: 809-820.
9. Chua, S.K., et al. 2016. Mechanical stretch inhibits microRNA499 via p53 to regulate calcineurin-A expression in rat cardiomyocytes. PLoS ONE 11: e0148683.

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.