SANTA CRUZ BIOTECHNOLOGY, INC.

Myc-Max 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. Nucl. 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. Zervos, A.S., et al. 1993. Mxi1, a protein that specifically interacts with Max to bind Myc-Max recognition sites. Cell 72: 223-232.

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

Myc-Max CONSENSUS OLIGONUCLEOTIDE: sc-2509

- binding site for Max-Max homodimers, Mad-Max heterodimers and Myc-Max heterodimers (3)

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5′— GGA  AGC  AGA  C CA  CGT  G GT  CTG  CTT  CC — 3′
3′— CCT  TCG  TCT  G GT  GCA  C CA  GAC  GAA  GG — 5′
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Myc-Max MUTANT OLIGONUCLEOTIDE: sc-2510

- identical to sc-2509 with the exception of a "TG"→"GA" substitution in the Myc-Max DNA binding motif (3)

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5′— GGA  AGC  AGA  C CA  CG G  A GT  CTG  CTT  CC — 3′
3′— CCT  TCG  TCT  G GT  GCC  T CA  GAC  GAA  GG — 5′
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SELECT PRODUCT CITATIONS

1. Kyo, S., et al. 2000. Sp1 cooperates with c-Myc to activate transcription of the human telomerase reverse transcriptase gene (hTERT). Nucleic Acids Res. 28: 669-677.
2. Goebel, J., et al. 2001. Stat5 pathway: target of anti-CD4 antibody in attenuation of IL-2 receptor signaling. Transplantation 71: 792-796.
3. 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.
4. Strazza, M., et al. 2004. Activation of cell cycle regulatory proteins in the apoptosis of terminally differentiated oligodendrocytes. Neurochem. Res. 29: 923-931.
5. Shyu, K.G., et al. 2005. Regulation of discoidin domain receptor 2 by cyclic mechanical stretch in cultured rat vascular smooth muscle cells. Hypertension 46: 614-621.
6. Han, S.S., et al. 2010. NF-κB/STAT3/PI3K signaling crosstalk in iMyc E µ B lymphoma. Mol. Cancer 9: 97.
7. Han, S.S., et al. 2013. Piperlongumine inhibits proliferation and survival of Burkitt lymphoma *in vitro*. Leukemia Res. 37: 146-154.
8. Han, S.S., et al. 2015. CDKN1A and FANCD2 are potential oncotargets in Burkitt lymphoma and multiple myeloma. Exp. Hematol. Oncol. 4: 9.

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.