SANTA CRUZ BIOTECHNOLOGY, INC.

Smad3/4 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. Dennler, S., et al. 1998. Direct binding of Smad3 and Smad4 to critical TGFβ-inducible elements in the promoter of human plasminogen activator inhibitor-type 1 gene. EMBO J. 17: 3091-3100.

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.

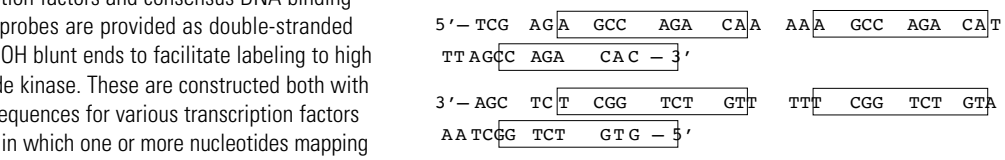
RESEARCH USE

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

PRODUCT

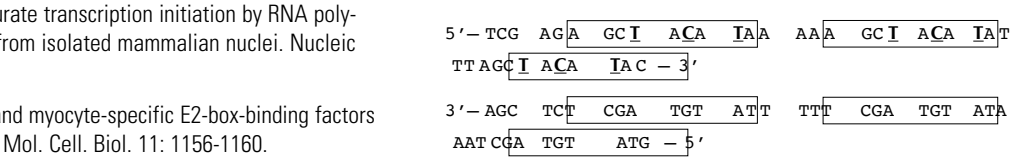
Smad3/4 CONSENSUS OLIGONUCLEOTIDE: sc-2597

- binding site for Smad3/4 transcription factor complex (3)



Smad3/4 MUTANT OLIGONUCLEOTIDE: sc-2598

- identical to sc-2597 with the exception of "C"→"T" and "G"→"C" substitutions in the CAGA binding motif (3)



SELECT PRODUCT CITATIONS

1. Poncelet, A.C. and Schnaper, H.W. 2001. Sp1 and Smad proteins cooperate to mediate transforming growth factor-β1-induced α2(I) collagen expression in human glomerular mesangial cells. J. Biol. Chem. 276: 6983-6992.
2. Hu, M.G., et al. 2004. Role of p12$^{CDK2-AP1}$ in transforming growth factor-β1-mediated growth suppression. Cancer Res. 64: 490-499.
3. Chow, E.K., et al. 2005. TLR agonists regulate PDGF-B production and cell proliferation through TGF-β/type I IFN crosstalk. EMBO J. 24: 4071-4081.
4. Luzina, I.G., et al. 2006. CCL18-stimulated upregulation of collagen production in lung fibroblasts requires Sp1 signaling and basal Smad3 activity. J. Cell. Physiol. 206: 221-228.
5. Gao, W., et al. 2009. Calcium signaling-induced Smad3 nuclear accumulation induces acetylcholinesterase transcription in apoptotic HeLa cells. Cell. Mol. Life Sci. 66: 2181-2193.
6. Gerjevic, L.N., et al. 2012. Alcohol activates TGF-β but inhibits BMP receptor-mediated Smad signaling and Smad4 binding to hepcidin promoter in the liver. Int. J. Hepatol. 2012: 459278.
7. Shyu, K.G., et al. 2013. Mechanical stretch via transforming growth factor-β1 activates microRNA208a to regulate endoglin expression in cultured rat cardiac myoblasts. Eur. J. Heart Fail. 15: 36-45.

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.