SANTA CRUZ BIOTECHNOLOGY, INC.

Smad SBE 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. Shi, Y., et al. 1998. Crystal structure of a Smad MH1 domain bound to DNA: insights on DNA binding in TGFβ signaling. Cell 94: 585-594.

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

Smad SBE CONSENSUS OLIGONUCLEOTIDE: sc-2603

- SBE binding site for Smad transcription factors (3)

5' – AGT ATG TCT AGA CTG A – 3'
3' – TCA TAC AGA TCT GAC T – 5'

Smad SBE MUTANT OLIGONUCLEOTIDE: sc-2604

- identical to sc-2603 with the exception of a "GTCTAGAC" → "CATAGCGT" substitution in the Smad SBE binding site (3)

5' – AGT ATC ATA GCG TTG A – 3'
3' – TCA TAG TAT CGC AAC T – 5'

SELECT PRODUCT CITATIONS

1. Maliekal, T.T., et al. 2004. Differential activation of Smads in HeLa and SiHa cells that differ in their response to transforming growth factor-β. J. Biol. Chem. 279: 36287-36292.
2. Akool, E.S., et al. 2005. Nitric oxide induces TIMP-1 expression by activating the transforming growth factor β-Smad signaling pathway. J. Biol. Chem. 280: 39403-39416.
3. Boyer Arnold, N. and Korc, M. 2005. Smad7 abrogates transforming growth factor-β1-mediated growth inhibition in COLO-357 cells through functional inactivation of the retinoblastoma protein. J. Biol. Chem. 280: 21858-21866.
4. Stuhlmeier, K.M. and Pollaschek, C. 2005. Adenovirus-mediated gene transfer of mutated IκB kinase and IκBα reveal NFκB-dependent as well as NFκB-independent pathways of HAS1 activation. J. Biol. Chem. 280: 42766-42773.
5. Liu, G., et al. 2006. Requirement of Smad3 and CREB-1 in mediating transforming growth factor-β (TGF β) induction of TGF β 3 secretion. J. Biol. Chem. 281: 29479-29490.
6. Cho, I.J., et al. 2006. Inhibition of TGFβ1-mediated PAI-1 induction by oltipraz through selective interruption of Smad 3 activation. Cytokine 35: 284-294.
7. Akool, el-S., et al. 2008. Molecular mechanisms of TGF β receptor-triggered signaling cascades rapidly induced by the calcineurin inhibitors cyclosporin A and FK506. J. Immunol. 181: 2831-2845.
8. Kaminski, S., et al. 2011. Coronin 1A is an essential regulator of the TGFβ receptor/SMAD3 signaling pathway in Th17 CD4+ T cells. J. Autoimmun. 37: 198-208.

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.