

Oct-1 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. Scheidereit, C., et al. 1988. A human lymphoid-specific transcription factor that activates immunoglobulin genes is a homeobox protein. *Nature* 336: 551-557.

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 [³²P]-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.

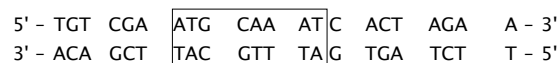
RESEARCH USE

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

PRODUCT

Oct-1 CONSENSUS OLIGONUCLEOTIDE: sc-2506

- binding site for Oct family homeodomain transcription factors (3)



Oct-1 MUTANT OLIGONUCLEOTIDE: sc-2515

- identical to sc-2506 with the exception of an "AT" → "GC" substitution in the Oct DNA binding motif (3)



SELECT PRODUCT CITATIONS

1. Jiang, J.G., et al. 1997. A novel transcriptional regulatory region within the core promoter of the hepatocyte growth factor gene is responsible for its inducibility by cytokines via the C/EBP family of transcription factors. *Mol. Cell. Biol.* 17: 5758-5770.
2. Ohata, T., et al. 1998. Inhibition by 1'-acetoxychavicol acetate of lipopolysaccharide- and interferon-gamma-induced nitric oxide production through suppression of inducible nitric oxide synthase gene expression in RAW264 cells. *Carcinogenesis* 19: 1007-1012.
3. Heltemes, L.M., et al. 1999. Tissue expression patterns of chicken octamer-binding proteins. *Poult. Sci.* 78: 574-578.
4. Fessele, S., et al. 2001. Molecular and in silico characterization of a promoter module and C/EBP element that mediate LPS-induced RANTES/CCL5 expression in monocytic cells. *FASEB J.* 15: 577-579.
5. Zhao, F.Q., et al. 2002. Involvement of Oct-1 in transcriptional regulation of β-casein gene expression in mouse mammary gland. *Biochim. Biophys. Acta* 1577: 27-37.
6. Chandrasekar, B., et al. 2003. Chemokine-cytokine cross-talk. The ELR+ CXC chemokine LIX (CXCL5) amplifies a proinflammatory cytokine response via a phosphatidylinositol 3-kinase-NFκB pathway. *J. Biol. Chem.* 278: 4675-4686.
7. Arguello, M., et al. 2003. Disruption of the B-cell specific transcriptional program in HHV-8 associated primary effusion lymphoma cell lines. *Oncogene* 22: 964-973.
8. Zhu, H., et al. 2007. The CCAAT-binding factor CBF/NF-Y regulates the human acetylcholinesterase promoter activity during calcium ionophore A23187-induced cell apoptosis. *Biochim. Biophys. Acta* 1770: 1475-1482.

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.