

# Pit-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. Yan, G.Z., et al. 1991. Thyrotropin-releasing hormone action on the prolactin promoter is mediated by the POU protein Pit-1. *Mol. Endoc.* 5: 535-541.

## GEL SHIFT ASSAYS

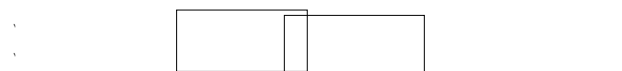
For gel shift analysis, prepare nuclear extracts following the method of Dignam, et al (1).

- **NOTE:** Spin oligonucleotide vial before opening. Product may be lodged in vial cap.
- Label oligonucleotide probe (TransCruz™ Gel Shift Oligonucleotides) with [ $\gamma$ -<sup>32</sup>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  $\mu$ l reaction mixture containing 3-10  $\mu$ g nuclear extract and 1  $\mu$ g 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  $\mu$ l of the appropriate TransCruz™ Gel Supershift antibody per 20  $\mu$ l 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

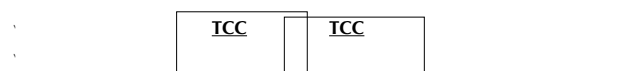
### Pit-1 CONSENSUS OLIGONUCLEOTIDE: sc-2541

- binding site for Pit-1 (3)



### Pit-1 MUTANT OLIGONUCLEOTIDE: sc-2542

- identical to sc-2541 with the exception of two "GAA" → "TCC" substitutions in the Pit-1 binding motif (3)



## SELECT PRODUCT CITATIONS

1. Shewchuk, B.M., et al. 1999. Pit-1 binding sites at the somatotrope-specific DNase I hypersensitive sites I, II of the human growth hormone locus control region are essential for *in vivo* hGH-N gene activation. *J. Biol. Chem.* 274: 35725-35733.
2. Lee, T.H., et al. 1999. Characterization of the murine gene encoding 1-Cys peroxiredoxin and identification of highly homologous genes. *Gene* 234: 337-344.
3. Dobretsova, A., et al. 2004. Potentiation of myelin proteolipid protein (Plp) gene expression is mediated through AP-1-like binding sites. *J. Neurochem.* 90: 1500-1510.
4. Sade, H., et al. 2009. Transcriptional control of occludin expression in vascular endothelia: regulation by Sp3 and YY1. *Biochim. Biophys. Acta* 1789: 175-184.
5. Palmieri, D., et al. 2012. Pit-1 upregulation by HMGA proteins has a role in pituitary tumorigenesis. *Endocr. Relat. Cancer* 19: 123-135.

## 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.

## PROTOCOLS

See our web site at [www.scbt.com](http://www.scbt.com) for detailed protocols and support products.