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HIF-1α Consensus and Mutant Oligonucleotides

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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. Mukhopadhyay, C.K., et al. 2000. Role of hypoxia-inducible factor-1 in transcriptional activation of ceruloplasmin by iron deficiency. J. Biol. Chem. 275: 21048-21054.

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

HIF-1α CONSENSUS OLIGONUCLEOTIDE: sc-2625

- binding site for HIF-1α transcription factor (3)

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5′– TCT  GTA  CGT  GAC  CAC  ACT  CAC  CTC – 3′
3′– AGA  CAT  GCA  CTG  GTG  TGA  GTG  GAG – 5′
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HIF-1α MUTANT OLIGONUCLEOTIDE: sc-2626

- identical to sc-2625 with the exception of a "CTG"→"AAA" substitution in the HIF-1α binding motif (3)

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5′– TCT  GTA  AAA  GAC  CAC  ACT  CAC  CTC – 3′
3′– AGA  CAT  TTT  CTG  GTG  TGA  GTG  GAG – 5′
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SELECT PRODUCT CITATIONS

1. Xi, L., et al. 2004. Cobalt chloride induces delayed cardiac preconditioning in mice through selective activation of HIF-1α and AP-1 and iNOS signaling. Am. J. Physiol. Heart Circ. Physiol. 287: H2369-H2375.
2. Hong, K.H., et al. 2005. Monocyte chemoattractant protein-1-induced angiogenesis is mediated by vascular endothelial growth factor-A. Blood 105: 1405-1407.
3. Kim, K.S. and Rajagopal, V. 2006. A novel role of hypoxia-inducible factor in cobalt chloride- and hypoxia-mediated expression of IL-8 chemokine in human endothelial cells. J. Immunol. 177: 7211-7224.
4. Chin, B.Y., et al. 2007. Hypoxia-inducible factor 1α stabilization by carbon monoxide results in cytoprotective preconditioning. Proc. Natl. Acad. Sci. USA 104: 5109-5114.
5. Patel, N., et al. 2008. Placenta growth factor augments endothelin-1 and endothelin-B receptor expression via hypoxia-inducible factor-1 α. Blood 112: 856-865.
6. Csak, T., et al. 2015. microRNA-122 regulates hypoxia-inducible factor-1 and vimentin in hepatocytes and correlates with fibrosis in diet-induced steatohepatitis. Liver Int. 35: 532-541.
7. Park, H., et al. 2015. 3,3'-diindolylmethane inhibits VEGF expression through the HIF-1α and NFκB pathways in human retinal pigment epithelial cells under chemical hypoxic conditions. Int. J. Mol. Med. 36: 301-308.

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.