SANTA CRUZ BIOTECHNOLOGY, INC.

NF-E2 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. Andrews, N.C., et al. 1993. Erythroid transcription factor NF-E2 is a haematopoietic-specific basic-leucine zipper protein. Nature 362: 722-728.

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

NF-E2 CONSENSUS OLIGONUCLEOTIDE: sc-2527

- binding site for erythroid transcription factor NF-E2 (3)

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5′– TGG  GGA  ACC  TGT  GCT  GAG  TCA  CTG  GAG – 3′
3′– ACC  CCT  TGG  ACA  CGA  CTC  AGT  GAC  CTC —5′
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NF-E2 MUTANT OLIGONUCLEOTIDE: sc-2528

- identical to sc-2527 with the exception of a "GA"→"AG" substitution in the DNA binding region (3)

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5′– TGG  GGA  ACC  TGT  GCT  AGG  TCA  CTG  GAG – 3′
3′– ACC  CCT  TGG  ACA  CGA  TCC  AGT  GAC  CTC —5′
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SELECT PRODUCT CITATIONS

1. Yamada, T., et al. 1998. Reduction of DNA binding activity of the GATA-1 transcription factor in the apoptotic process induced by overexpression of PU.1 in murine erythroleukemia cells. Exp. Cell Res. 245: 186-194.
2. Hayasaka, H., et al. 2005. FRNK, the autonomously expressed C-terminal region of focal adhesion kinase, is uniquely regulated in vascular smooth muscle: analysis of expression in transgenic mice. J. Cell. Biochem. 95: 1248-1263.
3. Min, K.J., et al. 2006. Astrocytes induce hemeoxygenase-1 expression in microglia: a feasible mechanism for preventing excessive brain inflammation. J. Neurosci. 26: 1880-1887.
4. Narasimhan, M., et al. 2011. Overexpression of Nrf2 protects cerebral cortical neurons from ethanol-induced apoptotic death. Mol. Pharmacol. 80: 988-999.
5. Lee, S., et al. 2012. An effective strategy for increasing the radiosensitivity of human lung cancer cells by blocking Nrf2-dependent antioxidant responses. Free Radic. Biol. Med. 53: 807-816.
6. Joo Choi, R., et al. 2014. Desoxyrhapontigenin up-regulates Nrf2-mediated heme oxygenase-1 expression in macrophages and inflammatory lung injury. Redox Biol. 2: 504-512.
7. Lee, E.J., et al. 2015. β-lapachone suppresses neuroinflammation by modulating the expression of cytokines and matrix metalloproteinases in activated microglia. J. Neuroinflammation 12: 133.
8. Sahni, A., et al. 2016. Bortezomib effects on human microvascular endothelium *in vitro*. Pharmacology 98: 272-278.
9. Shrishrimal, S., et al. 2020. Manganese porphyrin, MnTE-2-PyP, treatment protects the prostate from radiation-induced fibrosis (RIF) by activating the Nrf2 signaling pathway and enhancing SOD2 and sirtuin activity. Free Radic. Biol. Med. 152: 255-270.

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.