SANTA CRUZ BIOTECHNOLOGY, INC.

MEF-2 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. Martin, J.F., et al. 1993. Myocyte enhancer factor (MEF) 2C: a tissue-restricted member of the MEF-2 family of transcription factors. Proc. Natl. Acad. Sci. USA 90: 5282-5286.

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

MEF-2 CONSENSUS OLIGONUCLEOTIDE: sc-2521

- binding site for MEF-2, MEF-2C, xMEF-2 and RSRF transcription factors (3)

5' - GAT CGC TCT AAA AAT AAC CCT GTC G - 3'
3' - CTA GCG AGA TTT TTA TTG GGA CAG C - 5'

MEF-2 MUTANT OLIGONUCLEOTIDE: sc-2522

- identical to sc-2521 with the exception of "C"→"G" and "A"→"C" substitutions in the DNA binding motif (3)

5' - GAT CGC TGT AAA CAT AAC CCT GTC G - 3'
3' - CTA GCG ACA TTT GTA TTG GGA CAG C - 5'

SELECT PRODUCT CITATIONS

1. Nakayama, M., et al. 1996. Common core sequences are found in skeletal muscle slow and fast fiber type specific regulatory elements. Mol. Cell. Biol. 16: 2408-2417.
2. Kolodziejczyk, S.M., et al. 1999. MEF2 is upregulated during cardiac hypertrophy and is required for normal post-natal growth of the myocardium. Curr. Biol. 9: 1203-1206.
3. Mora, S., et al. 2000. The MEF-2A isoform is required for striated muscle-specific expression of the Insulin-responsive Glut4 glucose transporter. J. Biol. Chem. 275: 16323-16328.
4. Burton, T.R., et al. 2002. Anti-apoptotic wild-type Alzheimer amyloid precursor protein signaling involves the p38 mitogen-activated protein kinase/MEF2 pathway. Brain Res. Mol. Brain Res. 108: 102-120.
5. Chan, J.K., et al. 2003. Functional characterization of an amino-terminal region of HDAC4 that possesses MEF-2 binding and transcriptional repressive activity. J. Biol. Chem. 278: 23515-23521.
6. Nadruz, W., et al. 2003. Load-induced transcriptional activation of c-Jun in rat myocardium: regulation by myocyte enhancer factor 2. Circ. Res. 92: 243-251.
7. Nadruz, W., et al. 2005. Focal adhesion kinase mediates MEF2 and c-Jun activation by stretch: role in the activation of the cardiac hypertrophic genetic program. Cardiovasc. Res. 68: 87-97.
8. Shyu, K.G., et al. 2005. Insulin-like growth factor-1 mediates stretch-induced upregulation of myostatin expression in neonatal rat cardiomyocytes. Cardiovasc. Res. 68: 405-414.
9. Costelli, P., et al. 2007. Modulations of the calcineurin/NF-AT pathway in skeletal muscle atrophy. Biochim. Biophys. Acta 1770: 1028-1036.
10. Dwivedi, Y., et al. 2007. Aberrant extracellular signal-regulated kinase (ERK) 5 signaling in hippocampus of suicide subjects. Neuropsychopharmacology 32: 2338-2350.

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.