SANTA CRUZ BIOTECHNOLOGY, INC.

Egr Consensus and Mutant Oligonucleotides

BACKGROUND

Egr-1, Egr-2, Egr-3 and Egr-4 are nuclear transcription factors belonging to the Egr C_2H_2-type zinc-finger protein family and containing three C_2H_2-type zinc fingers. As immediate early proteins, Egr transcription factors are rapidly induced by diverse extracellular stimuli. They are subject to tight differential control through diverse mechanisms at several levels of regulation: transcriptional; translational and posttranslational (including glycosylation, phosphorylation and redox) mechanisms; and protein-protein interaction. Egr-1 binds to the DNA sequence 5'-CGCCCCCGC-3' (Egr-site), thereby activating transcription of target genes whose products are required for mitogenisis and differentiation. Egr-2 binds specific DNA sites located in the promoter region of HoxA4. Egr-2 defects cause congenital hypo-myelination neuropathy (also designated Charcot-Marie-Tooth disease) and Dejerine-Sottas neuropathology (also designated hereditary motor and sensory neuropathy III). Egr-3 is involved in muscle spindle development and is expressed in T cells 20 minutes following activation. Egr-4 binds to the Egr consensus motif GCGTGGGCG, functions as a transcriptional repressor, and displays autoregulatory activities, downregulating its own gene promoter in a dose dependent manner.

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

Egr CONSENSUS OLIGONUCLEOTIDE: sc-2529

- binding site for Egr transcription family (3)

5' - GGA TCC AGC GGG GGC GAG CGG GGG CGA - 3'
3' - CCT AGG TCG CCC CCG CTC GCC CCC GCT - 5'

Egr MUTANT OLIGONUCLEOTIDE: sc-2530

- identical to sc-2529 with the exception of a "GG"→"TA" substitution in the DNA binding regions (3)

5' - GGA TCC AGC TAG GGC GAG CGG GGG CGA - 3'
3' - CCT AGG TCG ATC CCG CTC GCC CCC GCT - 5'

SELECT PRODUCT CITATIONS

1. Tamaki, T., et al. 1995. Characterization of a GC-rich region containing Sp1 binding site(s) as a constitutive responsive element of the α 2(I) collagen gene in human fibroblasts. J. Biol. Chem. 270: 4299-4304.
2. Biesiada, E., et al. 1996. Egr-1 activates basic fibroblast growth factor transcription. Mechanistic implications for astrocyte proliferation. J. Biol. Chem. 271: 18576-18581.
3. Yan, S., et al. 1998. Tissue factor transcription driven by Egr-1 is a critical mechanism of murine pulmonary fibrin deposition in hypoxia. Proc. Natl. Acad. Sci. USA 95: 8298-8303.
4. Thottassery, J.V., et al. 1999. Sp1 and Egr-1 have opposing effects on the regulation of the rat Pgp2/Mdr1b gene. J. Biol. Chem. 274: 3199-3206.
5. Fu, M., et al. 2002. Early growth response factor-1 is a critical transcriptional mediator of peroxisome proliferator-activated receptor-γ 1 gene expression in human aortic smooth muscle cells. J. Biol. Chem. 277: 26808-26814.
6. Bradbury, D., et al. 2005. Vascular endothelial growth factor induction by prostaglandin E2 in human airway smooth muscle cells is mediated by E prostanoid EP2/EP4 receptors and Sp1 transcription factor binding sites. J. Biol. Chem. 280: 29993-30000.
7. Harju-Baker, S., et al. 2008. Silencing of Aγ-globin gene expression during adult definitive erythropoiesis mediated by GATA-1-FOG-1-Mi2 complex binding at the -566 GATA site. Mol. Cell. Biol. 28: 3101-3113.
8. Yoshida, Y., et al. 2009. Effects of 1-bromopropane, a substitute for chlorofluorocarbons, on BDNF expression. Int. Immunopharmacol. 9: 433-438.
9. Albrecht, C., et al. 2010. Egr-1 deficiency in bone marrow-derived cells reduces atherosclerotic lesion formation in a hyperlipidaemic mouse model. Cardiovasc. Res. 86: 321-329.

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.