SANTA CRUZ BIOTECHNOLOGY, INC.

NFκB Consensus and Mutant Oligonucleotide Agarose Conjugates

PRODUCT

Transcription factor consensus gel shift oligonucleotides containing specific consensus sequences and mutant control oligonucleotides are provided as agarose conjugates for use in purifying or enriching for specific transcription factors. TransCruz™ Oligonucleotide Agarose Conjugates are provided as 15 µg double-stranded oligonucleotide in 0.25 ml packed beads (1.0 ml total volume). Provides sufficient reagent for 10 assays.

NFκB CONSENSUS OLIGONUCLEOTIDE: sc-2505 AC

- binding site for NFκB/c-Rel homodimeric and heterodimeric complexes (3)

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5′— AGT  TGA  GGG  GAC  TTT  CCC  AGG  C —3′
3′—TCA  ACT  CCC  CTG  AAA  GGG  TCC  G —5′
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NFκB MUTANT OLIGONUCLEOTIDE: sc-2511 AC

- identical to sc-2505 with the exception of a "G"→"C"substitution in the NFκB/Rel DNA binding motif (3)

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5′— AGT  TGA  GG C  GAC  TTT  CCC  AGG  C —3′
3′—TCA  ACT  CCG  CTG  AAA  GGG  TCC  G —5′
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REFERENCES

1. Dignam, J.D., Lebovitz, R.M. and Roeder, R.G. 1983. Accurate transcription initiation by RNA polymerase II in a soluble extract from isolated mammalian nuclei. Nucl. Acids Res. 11: 1475-1489.
2. Murre, C., Voronova, A. and Baltimore, D. 1991. B cell- and myocyte-specific E2-box-binding factors contain E12/E47-like subunits. Mol. Cell. Biol. 11: 1156-1160.
3. Lenardo, M.J., et al. 1989. NFκB: a pleiotropic mediator of inducible and tissue-specific gene control. Cell 58: 227-229.

PREPARATION OF SOLUTIONS

- Binding buffer: 10 mM Tris, pH 7.5; 50 mM NaCl; 1 mM DTT; 1 mM EDTA; 5% glycerol; 1 µg/ml poly dI-dC.
- Elution buffer: Same as binding buffer, but increase NaCl concentration to 150 mM.

PROCEDURE

- Thoroughly mix oligonucleotide agarose conjugate slurry. Aliquot 100 µl slurry (containing 25 µl beads) into 1.5 ml microcentrifuge tube. To pellet beads, centrifuge at 12,000 rpm for 3-5 minutes in microcentrifuge at 4° C. Aspirate supernatant and wash pellet 3 times as follows: add 1 ml binding buffer, resuspend beads and centrifuge at 12,000 rpm for 3-5 minutes in microcentrifuge at 4° C, aspirating supernatant after each wash.
- To the washed agarose pellet, add 250-1000 µg nuclear extract or whole cell lysate (preferably <200 µl in volume). Add sufficient binding buffer to bring total volume to 500 µl. (If a large volume of extract/lysate is used, adjust final NaCl concentration to approximately 50 mM. Alternatively, extract/lysate can be prepared in binding buffer.)
- Incubate with rotation for 2 hours at room temperature or overnight at 4° C. Centrifuge at 12,000 rpm for 3-5 minutes in microcentrifuge at 4° C and aspirate supernatant. Wash pellet 3 times with binding buffer as described above.
- To elute protein from washed beads, add 250 µl elution buffer and incubate for 30 minutes with rotation at room temperature. Centrifuge at 12,000 rpm for 3-5 minutes at 4° C to pellet beads.
- Carefully collect supernatant; this is the protein sample. If desired, concentrate protein sample using a commercially available micro-concentrator.
- To 10-25 µl concentrated sample, add an equal volume of SDS-PAGE electrophoresis sample buffer. Boil for 90 seconds.
- Analyze by Western blot analysis (loading up to 20 µl per lane) or other suitable research application according to Santa Cruz Biotechnology, Inc. research applications protocols.

ALTERNATE PROCEDURE

- Complete preparation and incubation of sample as in steps 1-3 above.
- Directly add 20-50 µl SDS-PAGE electrophoresis sample buffer to the washed pellet. Boil for 90 seconds. Centrifuge at 12,000 rpm for 3-5 minutes in microcentrifuge at 4° C.
- Analyze supernatant by Western blot analysis as in final step above.

SELECT PRODUCT CITATIONS

1. Peng, H.B., et al. 1995. Induction and stabilization of IκB-α by nitric oxide mediates inhibition of NFκB. J. Biol. Chem. 270: 14214-14219.
2. Breithaupt, T.B., et al. 1996. The suppression of T cell function and NFκB expression by Serine protease inhibitors is blocked by N-acetylcysteine. Cell. Immunol. 173: 124-130.
3. Barbeau, B., et al. 1997. Activation of HIV-1 long terminal repeat transcription and virus replication via NFκB dependent and independent pathways by potent phosphotyrosine phosphatase inhibitors, the peroxovanadium compounds. J. Biol. Chem. 272: 12968-12977.
4. Bourcier, T., et al. 1997. The NFκB signaling pathway participates in dysregulation of vascular smooth muscle cells *in vitro* and in human atherosclerosis. J. Biol. Chem. 272: 15817-15824.
5. Xia, Y., et al. 1997. RelB regulation of chemokine expression modulates local inflammation. Am. J. Pathol. 151: 375-387.
6. Kim, I.Y., et al. 1997. Inhibition of NFκB DNA binding and nitric oxide induction in human T cells and lung adenocarcinoma cells by selenite treatment Proc. Natl. Acad. Sci. USA 94: 12904-12907.

STORAGE

Store at 4° C; stable for one year from the date of shipment.

RESEARCH USE

For research use only, not for use in diagnostic procedures.