SANTA CRUZ BIOTECHNOLOGY, INC.

p-NFκB p65 (Thr 254): sc-101753

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The Power to Question

BACKGROUND

Proteins encoded by the v-Rel viral oncogene and its cellular homolog, c-Rel, are members of a family of transcription factors that include the two subunits of the transcription factor NFκB (p50 and p65) and the *Drosophila* maternal morphogen, Dorsal. Both proteins specifically bind to DNA sequences that are the same or slight variations of the 10 bp kB sequence in the immunoglobulin k light chain enhancer. This same sequence is also present in a number of other cellular and viral enhancers. The DNA binding activity of NFκB is activated and NFκB is subsequently transported from the cytoplasm to the nucleus in cells exposed to mitogens or growth factors. cDNAs encoding precursors for two distinct proteins have been described, designated p105 and p100. The p105 precursor contains p50 at its N-terminus and a C-terminal region that when expressed as a separate molecule, designated PDI, binds to p50 and regulates its activity. The NFκB transcription factor is a protein complex consisting of a DNA binding subunit and an associated protein. The DNA binding subunit, also referred to as Rel A, is functionally related to c-Rel p75 and RelB p68. NFκB p65 is phosphorylated at Serine 311 as a response to protein kinase C ζ

REFERENCES

1. Meyer, R., et al. 1991. Cloning of the DNA binding subunit of human NFκB: the level of its mRNA is strongly regulated by phorbol ester or tumor necrosis factor α. Proc. Natl. Acad. Sci. USA 88: 966-970.
2. Schmid, R.M., et al. 1991. Cloning of an NFκB subunit which stimulates HIV transcription in synergy with p65. Nature 352: 733-736.
3. Perkins, N.D., et al. 1992. Distinct combinations of NFκB subunits determine the specificity of transcriptional activation. Proc. Natl. Acad. Sci. USA 89: 1529-1533.
4. Ballard, D.W., et al. 1992. The 65 kDa subunit of human NFκB functions as a potent transcriptional activator and a target for v-Rel-mediated repression. Proc. Natl. Acad. Sci. USA 89: 1875-1879.
5. Hatada, E.N., et al. 1992. The Ankyrin repeat domains of the NFκB precursor p105 and the proto-oncogene Bcl-3 act as specific inhibitors of NFκB DNA binding. Proc. Natl. Acad. Sci. USA 89: 2489-2493.
6. Vermeulen, L., et al. 2003. Transcriptional activation of the NFκB p65 subunit by mitogen- and stress-activated protein kinase-1 (MSK1). EMBO J. 22: 1313–1324.

CHROMOSOMAL LOCATION

Genetic locus: RELA (human) mapping to 11q13.1; Rela (mouse) mapping to 19 A.

SOURCE

p-NFκB p65 (Thr 254) is a rabbit polyclonal antibody raised against a short amino acid sequence containing phosphorylated Thr 254 of NFκB p65 of human origin.

STORAGE

Store at 4° C, **DO NOT FREEZE**. Stable for one year from the date of shipment. Non-hazardous. No MSDS required.

PRODUCT

Each vial contains 100 µg IgG in 1.0 ml of PBS with < 0.1% sodium azide and 0.1% gelatin.

APPLICATIONS

p-NFκB p65 (Thr 254) is recommended for detection of Thr 254 phosphorylated NFκB p65 of mouse, rat and human origin by Western Blotting (starting dilution 1:200, dilution range 1:100-1:1000), immunoprecipitation [1–2 µg per 100–500 µg of total protein (1 ml of cell lysate)], immunofluorescence and immunohistochemistry (including paraffin-embedded sections) (starting dilution 1:50, dilution range 1:50-1:500).

Suitable for use as control antibody for NFκB p65 siRNA (h): sc-29410, NFκB p65 siRNA (h2): sc-44212 and NFκB p65 siRNA (m): sc-29411.

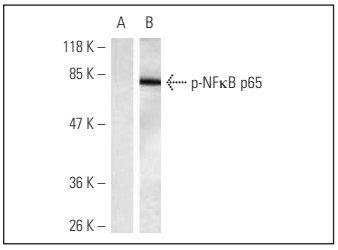
Molecular Weight of p-NFκB p65: 65 kDa.

Positive Controls: TNFα + Calyculin A-treated HT-29 whole cell lysate or human breast carcinoma tissue.

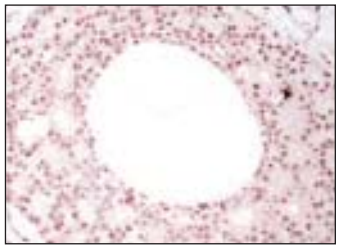
RECOMMENDED SECONDARY REAGENTS

To ensure optimal results, the following support (secondary) reagents are recommended: 1) Western Blotting: use goat anti-rabbit IgG-HRP: sc-2004 (dilution range: 1:2000-1:100,000) or Cruz Marker™ compatible goat anti-rabbit IgG-HRP: sc-2030 (dilution range: 1:2000-1:5000), Cruz Marker™ Molecular Weight Standards: sc-2035, TBS Blotto B Blocking Reagent: sc-2335 (use 50 mM NaF, sc-24988, as diluent) and Western Blotting Luminol Reagent: sc-2048. 2) Immunoprecipitation: use Protein A/G PLUS-Agarose: sc-2003 (0.5 ml agarose/2.0 ml). 3) Immunofluorescence: use goat anti-rabbit IgG-FITC: sc-2012 (dilution range: 1:100-1:400) or goat anti-rabbit IgG-TR: sc-2780 (dilution range: 1:100-1:400) with UltraCruz™ Mounting Medium: sc-24941. 4) Immunohistochemistry: use ImmunoCruz™: sc-2051 or ABC: sc-2018 rabbit IgG Staining Systems.

DATA



Western blot analysis of phosphorylated NFκB p65 expression in TNFα + Calyculin A-treated HT-29 whole cell lysate (**A**,**B**). Antibodies tested include p-NFκB p65 (Thr 254): sc-101753 preincubated with cognate phosphorylated peptide (**A**) and p-NFκB p65 (Thr 254): sc-101753 (**B**).



p-NFκB p65 (Thr 254): sc-101753. Immunoperoxidase staining of formalin-fixed, paraffin-embedded human breast carcinoma tissue showing nuclear staining.

RESEARCH USE

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