25 years of NF-κB at Rockland

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OVERVIEW

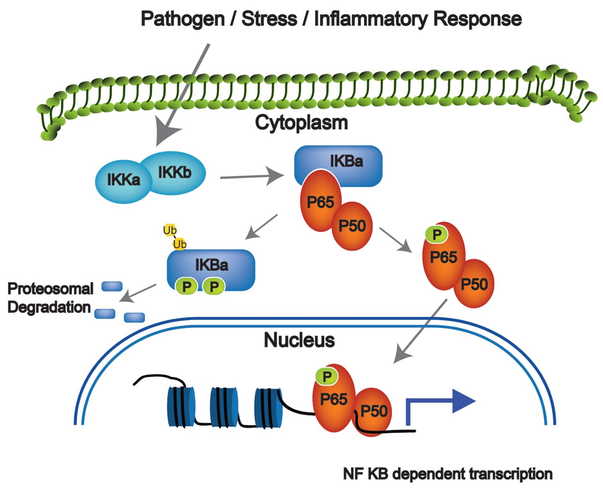
More than two decades ago David Baltimore and Ranjan Sen discovered a DNA binding factor that was found to be ubiquitously present and conserved in several biochemical pathways. Twenty-five years later, more than 44,000 articles have been published on NF-κB and its partners. To mark the 25th anniversary of the discovery of NF-κB we look at the antibodies we produced against NF-κB, its phosphorylated forms, subunits, inhibitors and NLS sites using new technologies. At Rockland, our philosophy is to continuously reinvent ourselves and deliver the best antibodies and tools to target NF-κB and its related proteins.

ORIGINS & DESCRIPTION OF NF-kB

In the early 80's, while working on the elucidation of immunoglobulin κ-chain gene regulation mechanism at the David Baltimore laboratory (MIT), Ranjan Sen isolated DNA binding factors through electrophoretic mobility-shift assay (EMSA). These factors were involved in broad biological activities including, octamer binding proteins in stem cell biology, basic helix-loop-helix proteins in lymphopoiesis and basic helix-loop-helix zipper proteins with the protooncoprotein c-Myc. Among these DNA binding proteins, NF-κB was described as a nucleoprotein complex localized on the κ3 fragment of κE.

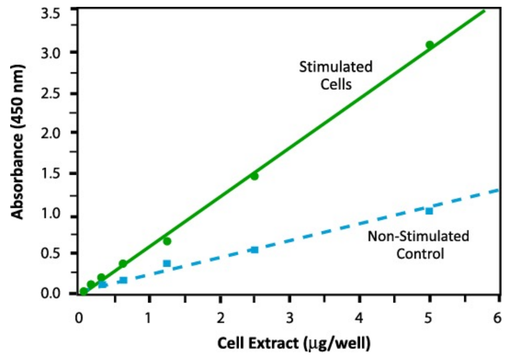
NF-κB (nuclear factor κ-light-chain-enhancer of activated B cells) is a protein complex that controls the transcription of DNA. NF-κB is found in almost all animal cell types and is involved in cellular responses to stimuli such as stress, cytokines, free radicals, ultraviolet irradiation, oxidized LDL and bacterial or viral antigens. More recently, NF-κB has been linked to cancer, inflammatory and autoimmune diseases, septic shock, viral infection, and improper immune development. Therefore, NF-κB represents a key target for drug discovery.

NF-kB SIGNALING & EFFECTORS



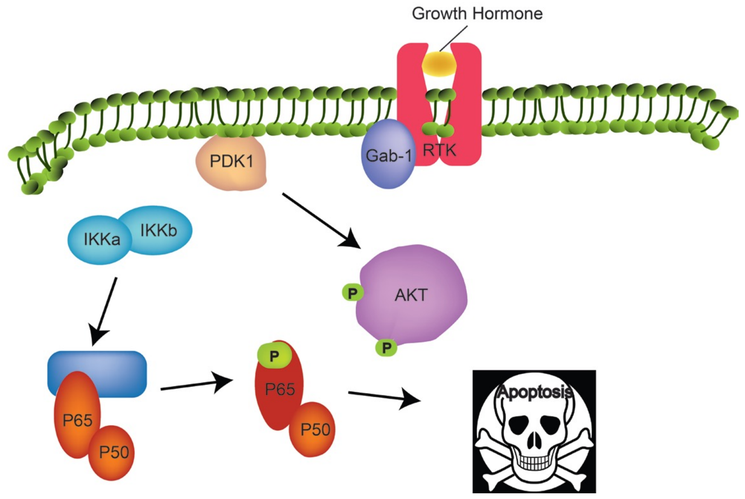
Schematic diagram 1: The NF-κB p65/p50 heterodimer is sequestered in the cytoplasm unless activated. Activating stimuli lead to ubiquitinylation and degradation of the IκB regulatory proteins which allows phosphorylation of the p65 subunit and translocation of the NF-κB dimer into the nucleus. Nuclear phosphorylated p65 can activate transcription of multiple immunomodulatory genes.

NF-kB TRANSCRIPTION FACTOR ASSAY



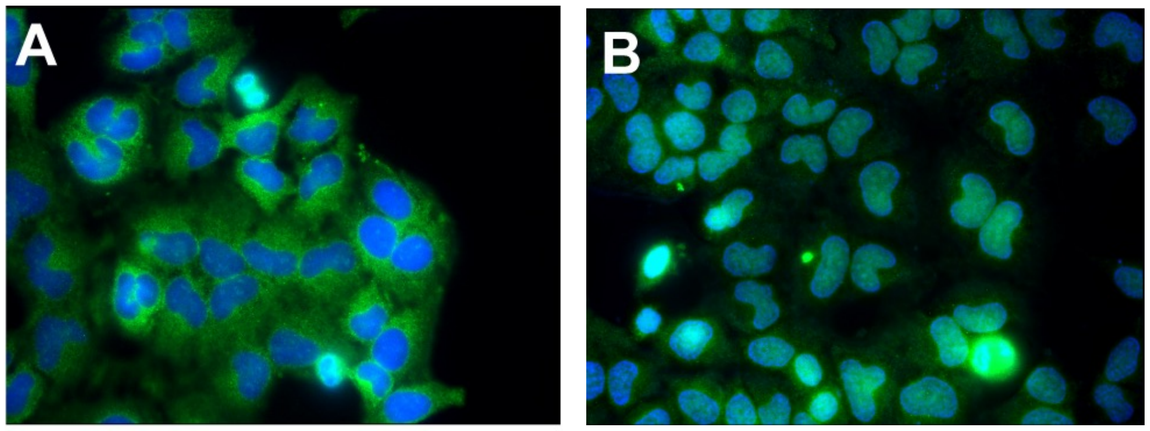
Anti-NF-κB (p65) Transcription Factor Assay. Transcription factor assay of stimulated cell lysate (20 ng / mL TNFα for 30 min) and non-stimulated lysate from HeLa cells demonstrating NF-κB (p65) activity. Rockland's NF-κB (p65) Transcription Factor Assay (KAA065) is a non-radioactive, sensitive method for detecting specific transcription factor DNA binding activity in nuclear extracts and whole cell lysates. Rockland's NF-κB (p65) Transcription Factor Assay detects human NF-κB (p65). A secondary antibody conjugated to peroxidase is added providing a sensitive colorimetric readout at 450 nm. Rockland's NF-κB (p65) Transcription Factor Assay detects human NF-κB (p65) and does not cross-react with NF-κB (p50).

NF-kB & CANCER



Schematic diagram 2: NF-κB is ubiquitously expressed in various cell types and its increased activity is associated with anti-apoptotic activity during cell transformation in cancer. In acute myeloid leukemia (AML), activation of PI3/ AKT kinases triggers an activity increase of NF-κB and its pro-anti-apoptotic effect. Understanding the basis of gene selection by NF-κB and the selectivity of its activation / inhibition by PI3-kinase / AKT will greatly facilitate the development of drugs in cancer therapy.

NF-kB IMMUNOFLUORESCENCE MICROSCOPY

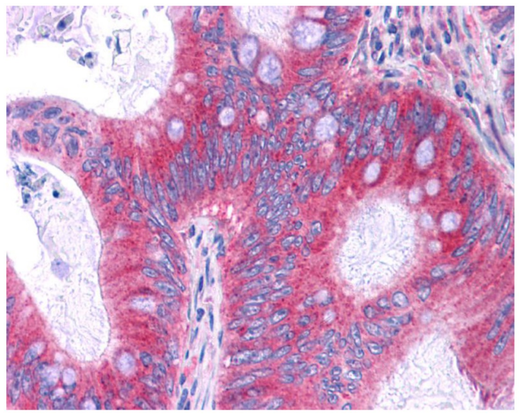


Immunofluorescence assay for p65 antibody. Rockland Monoclonal anti NF-κB p65 (Rel A) antibody was used to detect p65 by immunofluorescence. Subconfluent HeLa cell cultures were grown on 18 mm2 glass coverslips. Cells were either unstimulated, or stimulated with 50 ng / mL of TNFα for 30 min prior to fixation. Cells were then fixed in methanol and blocked with 10% normal goat serum (NGS) with 0.2% Triton detergent. Primary antibody 200-301-065 (clone number 33G2.F7.H8) was incubated at 1:1000 for 1 hr and then washed in blocking buffer. The secondary antibody was DyLight™ 488 conjugated goat anti mouse (p/n 610-141-121) at a dilution of 1:5000. Data were collected on a Leica STED-CW TCS-SP5 confocal microscope equipped with a DFC 350FX camera. Panel A shows cytoplasmic staining of p65 (green) in unstimulated HeLa cells. Panel B shows strong nuclear staining of p65 (green) in Hela. HeLa nuclei are stained with bis-benzimide stain (blue).

ROCKLAND NF-kB ANTIBODIES

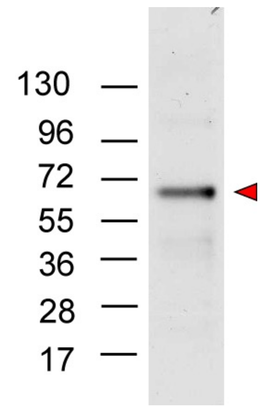
Our innovative team has developed the highest quality anti-NF-κB antibodies and antibodies to NF-κB subunits, antibodies to NF-κB inhibitors and antibodies to NF-κB activation sites for immunochemistry (IHC), transcription factor assays (EIA), gel super shift assays (GS), western-blot (WB) and immunofluorescence microscopy (IF). For more visit www.rockland.com.

NF-kB IMMUNOHISTOCHEMISTRY



Immunohistochemical assay for p65 antibody. Rockland Monoclonal Anti NF-κB (RelA) p/n 200-301-065 was tested in immunohistochemistry, analyzed by an anatomic pathologist, and validated for use in IHC applications against formalin-fixed tissues. Paraffin-embedded human tissues showed moderate to strong staining within many tissues, including epithelium of the breast, small intestine, kidney, pancreas, prostate, skin, placenta, thymus, and tonsil. The antibody produced an excellent signal with almost no background staining at a concentration of 2.5 µg/ml. The red signal shows specific staining in human colon carcinoma with a hematoxylin purple nuclear counterstain.

NF-kB WESTERN BLOT



Western Blot of NF-κB (p65). Rockland Monoclonal anti-NF-κB p65 antibody (RelA) p/n 200-301-065 is used to detect p65 by Western blot. Total cell lysate was prepared in RIPA lysis buffer (p/n MB-030-0050) and run on a 4-12% polyacrylamide gel and transferred onto a nitrocellulose membrane. The membrane was incubated with anti-p65 antibody at a dilution of 1:1,000 and detected with HRP conjugated anti-mouse secondary antibody at a dilution of 1:10,000. The blot shows detection of a specific band at 65 kDa corresponding to the RelA/p65 subunit of NF-κB.