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Anti-NF-kappaB p65 (Phospho-T254) Antibody

Catalog # AP60381

Product Information

Application	WB, IHC, IP, IF/IC
Primary Accession	Q04206
Reactivity	Human, Mouse, Rat, Pig, Bovine, Mk
Host	Rabbit
Clonality	Polyclonal
Calculated MW	60219

Additional Information

Gene ID	5970
Other Names	NFKB3; Transcription factor p65; Nuclear factor NF-kappa-B p65 subunit; Nuclear factor of kappa light polypeptide gene enhancer in B-cells 3
Target/Specificity	KLH-conjugated synthetic phosphopeptide corresponding to residues surrounding T254 of human NF-kappaB p65 protein. The exact sequence is proprietary.
Dilution	WB~~WB (1/500 - 1/1000), IHC (1/100 - 1/200), IP (1/10 - 1/100) IHC~~WB (1/500 - 1/1000), IHC (1/100 - 1/200), IP (1/10 - 1/100) IP~~N/A IF/IC~~N/A
Format	Liquid in 0.42% Potassium phosphate, 0.87% Sodium chloride, pH 7.3, 30% glycerol, and 0.01% sodium azide.
Storage	Store at -20 °C.Stable for 12 months from date of receipt

Protein Information

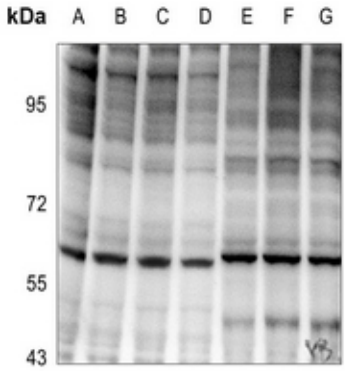
Name	RELA
Synonyms	NFKB3
Function	NF-kappa-B is a pleiotropic transcription factor present in almost all cell types and is the endpoint of a series of signal transduction events that are initiated by a vast array of stimuli related to many biological processes such as inflammation, immunity, differentiation, cell growth, tumorigenesis and apoptosis. NF-kappa-B is a homo- or heterodimeric complex formed by the Rel-like domain- containing proteins RELA/p65, RELB, NFKB1/p105, NFKB1/p50, REL and NFKB2/p52. The heterodimeric RELA-NFKB1 complex appears to be most abundant one. The dimers bind at kappa-B sites in the DNA of their target genes and the individual dimers have distinct preferences for different kappa-B sites that they can bind with distinguishable affinity and specificity. Different dimer combinations act as transcriptional activators or

repressors, respectively. The NF-kappa-B heterodimeric RELA-NFKB1 and RELA-REL complexes, for instance, function as transcriptional activators. NF-kappa-B is controlled by various mechanisms of post-translational modification and subcellular compartmentalization as well as by interactions with other cofactors or corepressors. NF-kappa-B complexes are held in the cytoplasm in an inactive state complexed with members of the NF-kappa-B inhibitor (I- kappa-B) family. In a conventional activation pathway, I-kappa-B is phosphorylated by I-kappa-B kinases (IKKs) in response to different activators, subsequently degraded thus liberating the active NF-kappa-B complex which translocates to the nucleus. The inhibitory effect of I- kappa-B on NF-kappa-B through retention in the cytoplasm is exerted primarily through the interaction with RELA. RELA shows a weak DNA- binding site which could contribute directly to DNA binding in the NF- kappa-B complex. Besides its activity as a direct transcriptional activator, it is also able to modulate promoters accessibility to transcription factors and thereby indirectly regulate gene expression. Associates with chromatin at the NF-kappa-B promoter region via association with DDX1. Essential for cytokine gene expression in T- cells (PubMed:15790681). The NF-kappa-B homodimeric RELA-RELA complex appears to be involved in invasin-mediated activation of IL-8 expression. Key transcription factor regulating the IFN response during SARS-CoV-2 infection (PubMed:33440148).

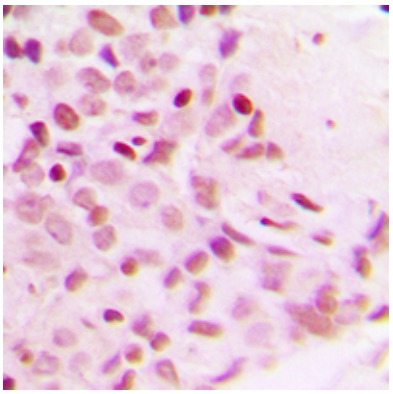
Cellular Location

Nucleus. Cytoplasm Note=Nuclear, but also found in the cytoplasm in an inactive form complexed to an inhibitor (I-kappa-B) (PubMed:1493333). Colocalized with DDX1 in the nucleus upon TNF induction (PubMed:19058135) Colocalizes with GFI1 in the nucleus after LPS stimulation (PubMed:20547752). Translocation to the nucleus is impaired in L.monocytogenes infection (PubMed:20855622)

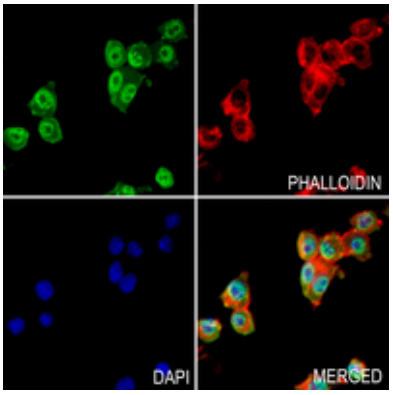
Images



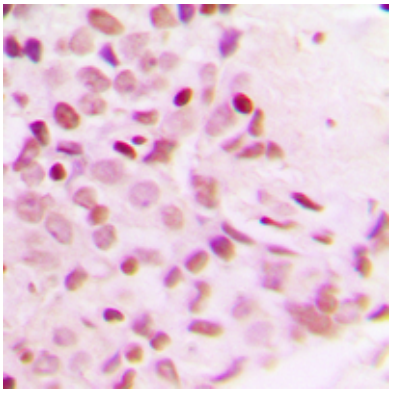
Western blot analysis of NF-kappaB p65 (Phospho-T254) expression in MCF7-LPS-30mins (A), MCF7-LPS-15mins (B), MCF7-LPS-5mins (C), MCF7 (D), Hela-TNFa-15mins (E), Hela-TNFa-5mins (F), Hela (G) whole cell lysates. (Predicted band size: 60 kD; Observed band size: 65 kD)



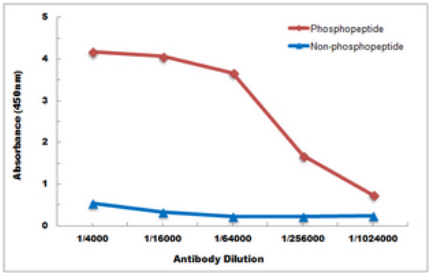
Immunohistochemical analysis of NF-kappaB p65 (Phospho-T254) staining in human breast cancer formalin fixed paraffin embedded tissue section. The section was pre-treated using heat mediated antigen retrieval with sodium citrate buffer (pH 6.0). The section was then incubated with the antibody at room temperature and detected using an HRP conjugated compact polymer system. DAB was used as the chromogen. The section was then counterstained with haematoxylin and mounted with DPX.



Immunofluorescent analysis of NF-kappaB p65 (Phospho-T254) staining in BV2 cells. Formalin-fixed cells were permeabilized with 0.1% Triton X-100 in TBS for 5-10 minutes and blocked with 3% BSA-PBS for 30 minutes at room temperature. Cells were probed with the primary antibody in 3% BSA-PBS and incubated overnight at 4 °C in a hidified chamber. Cells were washed with PBST and incubated with a AF488-conjugated secondary antibody (green) in PBS at room temperature in the dark. Phalloidin - AF594 was used to stain Actin filaments (red). DAPI was used to stain the cell nuclei (blue).



Immunohistochemical analysis of NF-kappaB p65 (pT254) staining in human breast cancer formalin fixed paraffin embedded tissue section. The section was pre-treated using heat mediated antigen retrieval with sodium citrate buffer (pH 6.0). The section was then incubated with the antibody at room temperature and detected using an HRP conjugated compact polymer system. DAB was used as the chromogen. The section was then counterstained with haematoxylin and mounted with DPX.



Direct ELISA antibody dose-response curve using Anti-NF-kappaB p65 (Phospho-T254) Antibody. Antigen (Phosphopeptide and non-phosphopeptide) concentration is 5 ug/ml. Goat Anti-Rabbit IgG (H&L) - HRP was used as the secondary antibody, and signal was developed by TMB substrate.

Please note: All products are 'FOR RESEARCH USE ONLY. NOT FOR USE IN DIAGNOSTIC OR THERAPEUTIC PROCEDURES'.