

**Anti-NFkB p65 NLS specific (RABBIT) Antibody**  
**NFkB p65 Antibody**  
**Catalog # ASR5184****Specification**

---

**Anti-NFkB p65 NLS specific (RABBIT) Antibody - Product Information**

|                  |                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                            |
|------------------|------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|
| Host             | Rabbit                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                     |
| Conjugate        | Unconjugated                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                               |
| Target Species   | Human                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                      |
| Reactivity       | Human                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                      |
| Clonality        | Polyclonal                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                 |
| Application      | WB, IHC, E, I, LCI                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                         |
| Application Note | Anti-NFkB p65 antibody has been tested in ELISA, ICC, WB, and IF. NFkB gel shift assays are assembled in 20 µl reactions containing 0.28 pmoles NFkB oligo in 10mM Tris (pH 7.6), 50 mM NaCl, 0.5 mM EDTA, 1.0 mM DTT, 10% glycerol. Some procedures specify the addition of 0.05% NP-40. When using purified protein, 250-300 ng should be sufficient to produce a gel shifted complex, while 10 µg HeLa nuclear extract is utilized. The gel shift reactions are then incubated at room temperature for 30 minutes. The complexes are resolved on Tris-Glycine acrylamide gels. Loading dye containing bromophenol blue and xylene cyanol should be added to the negative control reaction only, as these dyes can increase the dissociation of the NFkB complexes. When using HeLa nuclear extract as the source of binding proteins, two sequence-specific gel-shifted complexes are expected, consisting of p50/p50 homodimers and p50/p65 heterodimers. For cells expressing p52, p50, and p65, as many as four sequence-specific gel-shifted complexes could be observed (p52/p52, p50/p50, p52/p65, p50/p65), and if high levels of p65 are present, the p65/p65 homodimer may also be weakly detected. The following reagents have been observed to enhance NFkB binding in vitro: millimolar amounts of GTP and ATP, spermine, spermidine, barium or calcium ions, and µM amounts of Co+3(NH3)6. |
| Physical State   | Liquid (sterile filtered)                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                  |
| Buffer           | 0.02 M Potassium Phosphate, 0.15 M Sodium Chloride, pH 7.2                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                 |
| Immunogen        | This antibody was purified from whole                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                      |

Preservative

rabbit serum prepared by repeated immunizations with the NFkB p65 peptide corresponding to the NLS of the human protein conjugated to KLH using maleimide. A residue of cysteine was added to the amino terminal end to facilitate coupling.  
**0.01% (w/v) Sodium Azide**

### **Anti-NFkB p65 NLS specific (RABBIT) Antibody - Additional Information**

**Gene ID** 5970

**Other Names**  
5970

#### **Purity**

This affinity-purified antibody is directed against the nuclear localization sequence (NLS) NLS of human p65 and is useful in determining its presence in various assays. The epitope recognized overlaps the NLS of the p65 subunit of the NFkB heterodimer. Therefore, the antibody selectively binds to the activated form of NFkB. Anti-NFkB p65 NLS may react non-specifically with other proteins.

#### **Storage Condition**

Store vial at -20° C prior to opening. Aliquot contents and freeze at -20° C or below for extended storage. Avoid cycles of freezing and thawing. Centrifuge product if not completely clear after standing at room temperature. This product is stable for several weeks at 4° C as an undiluted liquid. Dilute only prior to immediate use.

#### **Precautions Note**

This product is for research use only and is not intended for therapeutic or diagnostic applications.

### **Anti-NFkB p65 NLS specific (RABBIT) Antibody - 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:<a href="http://www.uniprot.org/citations/15790681" target="\_blank">15790681</a>). 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:<a href="http://www.uniprot.org/citations/33440148" target="\_blank">33440148</a>).

### Cellular Location

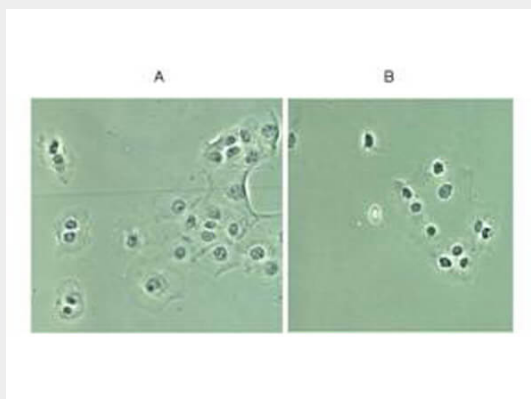
Nucleus. Cytoplasm. Note=Nuclear, but also found in the cytoplasm in an inactive form complexed to an inhibitor (I-kappa-B) (PubMed:1493333). Colocalized with DDX1 in the nucleus upon TNF-alpha induction (PubMed:19058135). Colocalizes with GFI1 in the nucleus after LPS stimulation (PubMed:20547752).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

### Anti-NFKB p65 NLS specific (RABBIT) Antibody - Protocols

Provided below are standard protocols that you may find useful for product applications.

- [Western Blot](#)
- [Blocking Peptides](#)
- [Dot Blot](#)
- [Immunohistochemistry](#)
- [Immunofluorescence](#)
- [Immunoprecipitation](#)
- [Flow Cytometry](#)
- [Cell Culture](#)

### Anti-NFKB p65 NLS specific (RABBIT) Antibody - Images



Rockland Immunochemical's Rabbit anti-p65 NLS was used at a 1:200 dilution to detect p65 in (A) control DU145 cells and (B) TNF stimulated DU145 cells. Although DU145 show relatively high basal levels of nuclear p65 staining, significant enhancement of nuclear staining is seen in panel B as evidence of translocation and availability of the NLS to be bound by the antibody. Cultured cells shown above were formalin-fixed. Tissue staining (not shown) were formalin-fixed, paraffin

embedded followed by citrate retrieval. Blocking and hybridization included 5% NGS.

#### **Anti-NFkB p65 NLS specific (RABBIT) Antibody - Background**

NFkB was originally identified as a factor that binds to the immunoglobulin kappa light chain enhancer in B cells. It was subsequently found in non-B cells in an inactive cytoplasmic form consisting of NFkB bound to IkB. NFkB was originally identified as a heterodimeric DNA binding protein complex consisting of p65 (RelA) and p50 (NFkB1) subunits. Other identified subunits include p52 (NFkB2), c-Rel, and RelB. The p65, cRel, and RelB subunits are responsible for transactivation. The p50 and p52 subunits possess DNA binding activity but limited ability to transactivate. p52 has been reported to form transcriptionally active heterodimers with the NFkB subunit p65, similar to p50/p65 heterodimers. Low levels of p52 and p50 homodimers can also exist in cells. The heterodimers of p52/p65 and p50/p65 are regulated by physical inactivation in the cytoplasm by IkB-a. IkB-a binds to the p65 subunit, preventing nuclear localization and DNA binding. IkB-a binding masks the NFkB nuclear localization signal (NLS). A broad range of external stimuli lead to activation of NFkB and set off signaling cascades that ultimately converge on the IkB kinase (IKK) complex. Activated IKK specifically and directly phosphorylates IkB-a and this phosphorylation event targets IkB-a for degradation. As a consequence, NFkB NLS is uncovered and nuclear translocation occurs.