NFkB Antibody Sampler Kit

Cat#: orb342330(User manual)

Physical State	Liquid (sterile filtered)
Label	Unconjugated
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.
Synonyms	Anti-NFKB p50, Anti-NFKB p65, Rabbit Anti-NFKB cRel, Anti-IKB alpha, Control peptides, antibody sample kits
Application Note	Each kit contains one unit of the following products: Anti-NFKB p50 (NFKB1) (RABBIT) Antibody (100 µL) Anti-NFKB p65 (Rel A) (RABBIT) Antibody (100 µL) Anti-NFKB cRel (RABBIT) Antibody (100 µL) Anti-IKBa C-terminal (RABBIT) Antibody (100 µL) Anti-RABBIT IgG (H&L) (GOAT) Peroxidase Conjugated Antibody (100 µg) CONTROL PEPTIDE for Anti-NFKB p50 (NFKB1) (RABBIT) Antibody (50 µg) CONTROL PEPTIDE for Anti-NFKB p65 (Rel A) (RABBIT) Antibody (50 µg) CONTROL PEPTIDE for Anti-NFKB cRel (RABBIT) Antibody (50 µg) CONTROL PEPTIDE for Anti-IKBa C-terminal (RABBIT) Antibody ((50 µg)
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. 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. Low levels of p52 and p50 homodimers can also exist in cells.

IKB-alpha inhibits the activity of dimeric NF-kappa-B/REL complexes by trapping REL dimers in the cytoplasm through masking of their nuclear localization signals. On cellular stimulation by immune and proinflammatory responses, IKBA becomes phosphorylated promoting ubiquitination and degradation, enabling the dimeric RELA to translocate to the nucleus and activate transcription.

Anti-NFkB and Anti-IKBa Antibodies are ideal for investigators involved in Cell Signaling, Neuroscience, Signal Transduction and Immunology research.

Assay Dilutions	User Optimized
ELISA	1:5,000 - 1: 25,000
Western Blot	1:500 - 1:2,000
Other Assays	User Optimized
Expiration	Expiration date is one (1) year from date of opening.

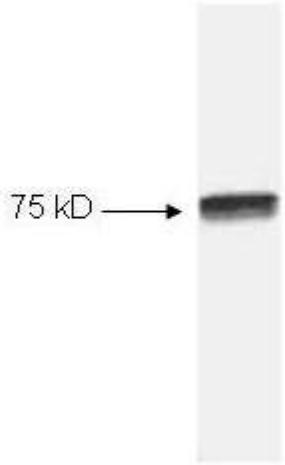
1. Western blot of HeLa cell extract.

A predominant band ~65 kDa (arrowhead) corresponding to NFkb p65 Rel A is observed with anti NFkB antibody 100-4165. All incubations except color development was performed using TBS supplemented with 0.1% Tween-20 at room temperature. The membrane was blocked in 5% dry milk for 2 h. After washing, a 1: 5,000 dilutions of the primary antibody were added to the membrane and incubated for 2 h. Washes with buffer were performed 4 times for 5' each. The western blot was incubated with secondary antibody (HRP Goat-a- Rabbit IgG [H&L]) diluted 1:2,000 for 1 h. Washes with TBS preceded color development.

2. Western blot of HeLa cell extract using p/n 100-4166 Anti-NFKB cRel (RABBIT) Antibody (100 µL).

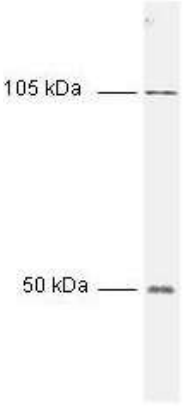
All incubations except color development was performed using TBS supplemented with 0.1% Tween-20 at room temperature. The membrane was blocked in 5% dry milk for 2 h. After washing, a:1: 1,000 dilutions of the

primary antibody were added to the membrane and incubated for 2 h. Washes with buffer were performed 4 times for 5' each. The western blot was incubated with secondary antibody (HRP Goat-a- Rabbit IgG [H&L]) diluted 1:2,000 for 1 h. Washes with TBS preceded color development.



3. Western blot of HeLa cell extract using Anti-NFKB p50 (NFKB1) Antibody 100-4164.

All incubations except color development were performed using TBS supplemented with 0.1% Tween-20 at room temperature. The membrane was blocked in 5% dry milk for 2 h. After washing, a:1: 1,000 dilutions of the primary antibody were added to the membrane and incubated for 2 h. Washes with buffer was performed 4 times for 5' each. The western blot was incubated with secondary antibody (HRP Goat-a-Rabbit IgG [H&L]) diluted 1:2,000 for 1 h. Washes with TBS preceded color development.



Disclaimer

This product is for research use only and is not intended for therapeutic or diagnostic applications. Please contact a technical service representative for more information.