**Product datasheet**

**NFkB p65 Transcription Factor Assay Kit ab133112**

**Overview**

- **Product name**: NFkB p65 Transcription Factor Assay Kit
- **Sample type**: Cell culture extracts, Nuclear Extracts
- **Assay type**: Semi-quantitative
- **Species reactivity**: Reacts with: Mouse, Rat, Human

**Product overview**

Abcam's NFkB p65 Transcription Factor Assay Kit (ab133112) is a non-radioactive, sensitive method for detecting specific transcription factor DNA binding activity in nuclear extracts. A 96-well enzyme-linked immunosorbent assay (ELISA) replaces the cumbersome radioactive electrophoretic mobility shift assay (EMSA). A specific double stranded DNA (dsDNA) sequence containing the NFkB response element is immobilized onto the bottom of wells of a 96-well plate. NFkB contained in a nuclear extract, binds specifically to the NFkB response element. NFkB (p65) is detected by addition of specific primary antibody directed against NFkB (p65). A secondary antibody conjugated to HRP is added to provide a sensitive colorimetric readout at 450 nm.

**Tested applications**

Suitable for: Functional Studies

**Properties**

**Storage instructions**

Please refer to protocols.

<table>
<thead>
<tr>
<th>Components</th>
<th>96 tests</th>
</tr>
</thead>
<tbody>
<tr>
<td>96-Well Plate Cover</td>
<td>1 unit</td>
</tr>
<tr>
<td>Polysorbate 20</td>
<td>1 vial</td>
</tr>
<tr>
<td>Transcription Factor Antibody Binding Buffer (10X)</td>
<td>1 x 3ml</td>
</tr>
<tr>
<td>Transcription Factor Binding Assay Buffer (4X)</td>
<td>1 x 3ml</td>
</tr>
<tr>
<td>Transcription Factor Developing Solution</td>
<td>1 x 12ml</td>
</tr>
<tr>
<td>Transcription Factor Goat Anti-Rabbit HRP Conjugate</td>
<td>1 x 100µl</td>
</tr>
<tr>
<td>Transcription Factor NFkB (Human p65) Positive Control</td>
<td>1 vial</td>
</tr>
</tbody>
</table>
NF-kappa-B is a pleiotropic transcription factor which is present in almost all cell types and is involved in 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 and the heterodimeric p65-p50 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. 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. NF-kappa-B heterodimeric p65-p50 and p65-c-Rel complexes are transcriptional activators. The NF-kappa-B p65-p65 complex appears to be involved in invasin-mediated activation of IL-8 expression. The inhibitory effect of I-kappa-B upon NF-kappa-B the cytoplasm is exerted primarily through the interaction with p65. p65 shows a weak DNA-binding site which could contribute directly to DNA binding in the NF-kappa-B complex. Associates with chromatin at the NF-kappa-B promoter region via association with DDX1.

Sequence similarities
Contains 1 RHD (Rel-like) domain.

Domain
the 9aaTAD motif is a transactivation domain present in a large number of yeast and animal transcription factors.

Post-translational modifications
Ubiquitinated, leading to its proteasomal degradation. Degradation is required for termination of NF-kappa-B response.
Monomethylated at Lys-310 by SETD6. Monomethylation at Lys-310 is recognized by the ANK repeats of EHMT1 and promotes the formation of repressed chromatin at target genes, leading to down-regulation of NF-kappa-B transcription factor activity. Phosphorylation at Ser-311 disrupts the interaction with EHMT1 without preventing monomethylation at Lys-310 and relieves the repression of target genes.
Phosphorylation at Ser-311 disrupts the interaction with EHMT1 and promotes transcription factor activity (By similarity). Phosphorylation on Ser-536 stimulates acetylation on Lys-310 and interaction with CBP; the phosphorylated and acetylated forms show enhanced transcriptional activity.
Reversibly acetylated; the acetylation seems to be mediated by CBP, the deacetylation by HDAC3. Acetylation at Lys-122 enhances DNA binding and impairs association with NFKBIA. Acetylation at Lys-310 is required for full transcriptional activity in the absence of effects on DNA binding.

<table>
<thead>
<tr>
<th>Components</th>
<th>Quantity</th>
</tr>
</thead>
<tbody>
<tr>
<td>Transcription Factor NFkB (p65) Primary Antibody</td>
<td>1 vial</td>
</tr>
<tr>
<td>Transcription Factor NF-kB 96-Well Strip Plate</td>
<td>1 unit</td>
</tr>
<tr>
<td>Transcription Factor NFkB Competitor dsDNA</td>
<td>1 vial</td>
</tr>
<tr>
<td>Transcription Factor Reagent A</td>
<td>1 x 120µl</td>
</tr>
<tr>
<td>Transcription Factor Stop Solution</td>
<td>1 x 12ml</td>
</tr>
<tr>
<td>Wash Buffer Concentrate (400X)</td>
<td>1 x 5ml</td>
</tr>
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**Components**

- **Transcription Factor NFkB (p65) Primary Antibody**: 1 vial
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**Function**

NF-kappa-B is a pleiotropic transcription factor which is present in almost all cell types and is involved in 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 and the heterodimeric p65-p50 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. 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. NF-kappa-B heterodimeric p65-p50 and p65-c-Rel complexes are transcriptional activators. The NF-kappa-B p65-p65 complex appears to be involved in invasin-mediated activation of IL-8 expression. The inhibitory effect of I-kappa-B upon NF-kappa-B the cytoplasm is exerted primarily through the interaction with p65. p65 shows a weak DNA-binding site which could contribute directly to DNA binding in the NF-kappa-B complex. Associates with chromatin at the NF-kappa-B promoter region via association with DDX1.

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binding and NFKBIA association. Acetylation can also lower DNA-binding and results in nuclear export. Interaction with BRMS1 promotes deacetylation of ‘Lys-310’.

**Cellular localization**

Nucleus. Cytoplasm. Nuclear, but also found in the cytoplasm in an inactive form complexed to an inhibitor (I-kappa-B). Colocalized with RELA in the nucleus upon TNF-alpha induction.

**Applications**

Our Abpromise guarantee covers the use of ab133112 in the following tested applications.

The application notes include recommended starting dilutions; optimal dilutions/concentrations should be determined by the end user.

<table>
<thead>
<tr>
<th>Application</th>
<th>Abreviews</th>
<th>Notes</th>
</tr>
</thead>
<tbody>
<tr>
<td>Functional Studies</td>
<td></td>
<td>Use at an assay dependent concentration. It does not cross-react with NFkB (p50) transcription factor.</td>
</tr>
</tbody>
</table>

**Images**

After the treatment with LPS (10 μg/ml for 6 hrs), cells were lysed with hypotonic HEPES lysis buffer (pH 7.4) and centrifuged at 1000 g for 10 min at 4°C, supernatants were collected and used for the determination of intracellular p65- NF-κB by ELISA. The absorbance was read at 450 nm using spectrophotometer.

Jurkat cells were treated with PMA and ionomycin (+). Nuclear lysates were extracted (ab113474) and 40 μL, corresponding to 4e6 cells, were tested in duplicates (+/- SD).

**Determining p65-NF-κB by ELISA using ab133112**

Saha D et al., PLoS One, 12(2). Fig 9a. doi: 10.1371/journal.pone.0171084
Functional Assay: ab133112 NFkB p65 Transcription Factor Assay Kit

Titration of positive control with or without inhibitor, background signal subtracted (duplicates; +/- SD).

Assay of cell lysates isolated from stimulated (20 ng/ml TNF alpha for 30 minutes) and nonstimulated HeLa cells demonstrating NFkB (p65) activity.

Please note: All products are "FOR RESEARCH USE ONLY AND ARE NOT INTENDED FOR DIAGNOSTIC OR THERAPEUTIC USE"

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