Product name: NF-kB p65/RelA Rabbit Monoclonal Antibody

Cat number: MAB22331
Conjugate: Unconjugated

Host: Rabbit Size: 100 ug

Synonyms: p65; CMCU; NFKB3; AIF3BL3; IA

Clone: C22B4
Concentration: 1mg/ml
Isotype: IgG

Immunogen: Synthetic peptide. This information is considered to be commercially

sensitive.

Reactivity: Human, Mouse, Rat, Monkey

Applications: WB 1:5000 - 1:20000 IHC-P 1:200 - 1:2000 IF/ICC 1:500 - 1:2000 IP

 $0.5\mu g$ - $4\mu g$ antibody for $200\mu g$ - $500\mu g$ extracts of whole cells ChIP $5\mu g$ antibody for $10\mu g$ - $15\mu g$ of Chromatin ELISA Recommended starting concentration is $1~\mu g/mL$. Please optimize the concentration based on

your specific assay requirements.

Molecular Weight: 65kDa

Purification: Affinity purification

Background: NF-kappa-B is a ubiquitous transcription factor involved in several

biological processes. It is held in the cytoplasm in an inactive state by specific inhibitors. Upon degradation of the inhibitor, NF-kappa-B moves to the nucleus and activates transcription of specific genes. NF-kappa-B is composed of NFKB1 or NFKB2 bound to either REL, RELA, or RELB. The most abundant form of NF-kappa-B is NFKB1 complexed with the product of this gene, RELA. Four transcript variants encoding different isoforms

have been found for this gene.

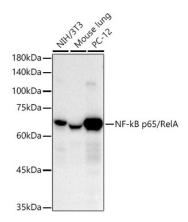
Form: liquid

Buffer: PBS containing 50% glycerol and 0.05% BSA, preserved with proclin300

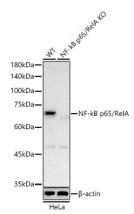
or sodium azide (as specified on the Certificate of Analysis), pH 7.3.

Storage: Store at -20°C. Avoid freeze / thaw cycles.

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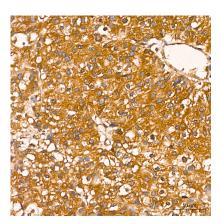


Western blot analysis of various lysates using [KO Validated] NF-kB p65/RelA Rabbit mAb at 1:10000 dilution. Secondary antibody: HRP-conjugated Goat anti-Rabbit IgG (H+L at 1:10000 dilution. Lysates/proteins: 25µg per lane. Blocking buffer: 3% nonfat dry milk in TBST. Detection: ECL West Pico Plus. Exposure time: 30s.



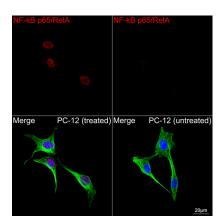
Western blot analysis of lysates from wild type(WT) and NF-kB p65/RelA knockout (KO)HeLa cells, using [KO Validated] NF-kB p65/RelA Rabbit mAb at 1:10000 dilution.Secondary antibody: HRP-conjugated Goat anti-Rabbit IgG (H+L) at 1:10000dilution.

Lysates/proteins: 25µg per lane. Blocking buffer: 3% nonfat dry milk in TBST.Detection: ECL West Pico Plus. Exposure time: 10s.

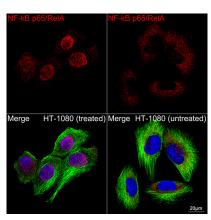


Immunohistochemistry analysis of paraffin-embedded Human liver tissue using [KO Validated] NF-kB p65/RelA Rabbit mAb at dilution of 1:200 (40x lens). High pressure antigen retrieval performed with 0.01M Citrate Buffer (pH 6.0) prior to IHC staining.

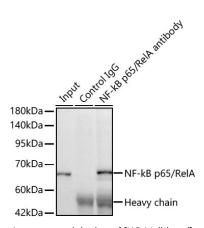
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Confocal imaging of PC-12 cells (treated with TNF-α) and PC-12 cells (untreated) cells using [KO Validated] NF-kB p65/RelA Rabbit mAb (, dilution 1:2000) followed by a further incubation with Cy3 Goat Anti-Rabbit IgG (H+L) (dilution 1:500) (Red). The cells were counterstained with α-Tubulin Mouse mAb (dilution 1:400) followed by incubation with Alexa Fluor 488-conjugated Goat Anti-Mouse IgG (H+L) Ab dilution 1:500) (Green). DAPI was used for nuclear staining (Blue). Objective: 100x.

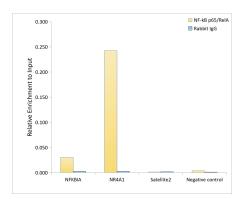


Confocal imaging of HT-1080 cells (treated with TNF-α) and HT-1080 cells (untreated) cells using [KO Validated] NF-kB p65/RelA Rabbit mAb (dilution 1:2000) followed by a further incubation with Cy3 Goat Anti-Rabbit IgG (H+L) (dilution 1:500) (Red). The cells were counterstained with α-Tubulin Mouse mAb (, dilution 1:400) followed by incubation with Alexa Fluor 488-conjugated Goat Anti-Mouse IgG (H+L) Ab (dilution 1:500) (Green). DAPI was used for nuclear staining (Blue). Objective: 100x.



Immunoprecipitation of [KO Validated]
NF-kB p65/RelA Rabbit mAb from 500
µg extracts of HeLa cells was
performed using 2 µg of [KO Validated]
NF-kB p65/RelA Rabbit mAb. Rabbit
IgG isotype control was used to
precipitate the Control IgG sample. IP
samples were eluted with 1X Laemmli
Buffer. The Input lane represents 10%
of the total input. Western blot analysis
of immunoprecipitates was conducted
using [KO Validated] NF-kB p65/RelA
Rabbit mAb at a dilution of 1:10000.

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Chromatin immunoprecipitation analysis of extracts of HT-1080 cells, HT-1080 cells were treated by TNF-α (20 ng/ml) at 37°C for 30 minutes, using [KO Validated] NF-kB p65/RelA Rabbit mAb and rabbit IgG.The amount of immunoprecipitated DNA was checked by quantitative PCR. Histogram was constructed by the ratios of the immunoprecipitated DNA to the input.

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