NF-κB p65 (D14E12) XP® Rabbit mAb

**Background:** Transcription factors of the nuclear factor κB (NF-κB)/Rel family play a pivotal role in inflammatory and immune responses (1,2). There are five family members in mammals: RelA, c-Rel, RelB, NF-κB1 (p105/p50), and NF-κB2 (p100/p52). Both p105 and p100 are proteolytically processed by the proteasome to produce p50 and p52, respectively. Rel proteins bind p50 and p52 to form dimeric complexes that bind DNA and regulate transcription. In unstimulated cells, NF-κB is sequestered in the cytoplasm by IκB inhibitory proteins (3-5). NF-κB-activating agents can induce the phosphorylation of IκB proteins, targeting them for rapid degradation through the ubiquitin-proteasome pathway and releasing NF-κB to enter the nucleus where it regulates gene expression (9-11).

**Specificity/Sensitivity:** NF-κB p65 (D14E12) XP® Rabbit mAb recognizes endogenous levels of total NF-κB p65/RelA protein. It does not cross react with other NF-κB/Rel family members.

**Source/Purification:** Monoclonal antibody is produced by immunizing animals with a synthetic peptide corresponding to residues surrounding Glu498 of human NF-κB p65/RelA protein.

**Applications**

- Western
- IP
- IHC-P
- IF-IC
- F
- ChIP
- ChIP-seq
- Endogenous

**Species Cross-Reactivity**

- H, M, R, Hm, Mk, Dg

**Molecular Wt.**

- 65 kDa

**Isotype**

- Rabbit IgG**

**Background References:**


**Recommended Antibody Dilutions:**

- Western blotting: 1:1000
- Immunoprecipitation: 1:100
- Immunohistochemistry (Paraffin): 1:400-1:1600
- Unmasking buffer: Citrate Antobody diluent: SignalStain® Antibody Diluent #8112
- Detection reagent: SignalStain® Boost (HRP, Rabbit) #8114
- Optimal IHC dilutions determined using SignalStain® Boost IHC Detection Reagent.
- Immunofluorescence (IF-IC): 1:400-1:1600
- Chromatin IP / Chromatin IP-seq: 1:100
- Optimal ChIP / ChIP-seq conditions: 5 µl of antibody & 20 µg of chromatin (4 X 10⁶ cells) per IP. Antibody validated using SimpleChIP® Enzymatic ChIP Kits.
- Flow Cytometry: 1:400-1:1600

For application specific protocols please see the web page for this product at www.cellsignal.com.

Please visit www.cellsignal.com for a complete listing of recommended companion products.

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Chromatin immunoprecipitations were performed with cross-linked chromatin from HeLa cells treated with hTNF-α #8902 (30 ng/ml, 1 hr) and either NF-κB p65 (D14E12) XP® Rabbit mAb or Normal Rabbit IgG #2729 using SimpleChIP® Enzymatic Chromatin IP Kit (Magnetic Beads) #9003. The enriched DNA was quantified by Real-Time PCR using SimpleChIP® Human IκBα Promoter Primers #5552, human IL-8 promoter primers, and SimpleChIP® Human α Satellite Repeat Primers #4486. The amount of immunoprecipitated DNA in each sample is represented as signal relative to the total amount of input chromatin, which is equivalent to one.

Chromatin immunoprecipitations were performed with cross-linked chromatin from HeLa cells treated with hTNF-α #8902 (30 ng/ml, 1 hr) and NF-κB p65 (D14E12) XP® Rabbit mAb, using SimpleChIP® Plus Enzymatic Chromatin IP Kit (Magnetic Beads) #9005. DNA Libraries were prepared using SimpleChIP® ChIP-seq DNA Library Prep Kit for Illumina® #56795. The figure shows binding across chromosome 4 (upper), including IL-8 (lower), a known target gene of NFκB (see additional figure containing ChIP-qPCR data).