

**#8242** Store at -20C

# NF- $\kappa$ B p65 (D14E12) XP<sup>®</sup> Rabbit mAb



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Applications:	Reactivity:	Sensitivity:	MW (kDa):	Source/Isotype:	UniProt ID:	Entrez-Gene Id:
W, W-S, IP, IHC-P, IF-IC, FC-FP, ChIP, ChIP-seq, C&R	H M R Hm Mk Dg	Endogenous	65	Rabbit IgG	#Q04206	5970

## Product Usage Information

For optimal ChIP and ChIP-seq results, use 5  $\mu$ l of antibody and 10  $\mu$ g of chromatin (approximately 4 x 10<sup>6</sup> cells) per IP. This antibody has been validated using SimpleChIP<sup>®</sup> Enzymatic Chromatin IP Kits.

The CUT&RUN dilution was determined using CUT&RUN Assay Kit #86652.

### Application

Application	Dilution
Western Blotting	1:1000
Simple Western™	1:10 - 1:50
Immunoprecipitation	1:100
Immunohistochemistry (Paraffin)	1:400 - 1:1600
Immunofluorescence (Immunocytochemistry)	1:400 - 1:800
Flow Cytometry (Fixed/Permeabilized)	1:400 - 1:1600
Chromatin IP	1:100
Chromatin IP-seq	1:100
CUT&RUN	1:100

## Storage

Supplied in 10 mM sodium HEPES (pH 7.5), 150 mM NaCl, 100  $\mu$ g/ml BSA, 50% glycerol and less than 0.02% sodium azide. Store at -20°C. Do not aliquot the antibody.

For a carrier free (BSA and azide free) version of this product see product #69994.

## Specificity/Sensitivity

NF- $\kappa$ B p65 (D14E12) XP<sup>®</sup> Rabbit mAb recognizes endogenous levels of total NF- $\kappa$ B p65/RelA protein. It does not cross react with other NF- $\kappa$ B/Rel family members.

## Source / Purification

Monoclonal antibody is produced by immunizing animals with a synthetic peptide corresponding to residues surrounding Glu498 of human NF- $\kappa$ B p65/RelA protein.

## Background

Transcription factors of the nuclear factor  $\kappa$ B (NF- $\kappa$ B)/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- $\kappa$ B1 (p105/p50), and NF- $\kappa$ B2 (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- $\kappa$ B is sequestered in the cytoplasm by I $\kappa$ B inhibitory proteins (3-5). NF- $\kappa$ B-activating agents can induce the phosphorylation of I $\kappa$ B proteins, targeting them for rapid degradation through the ubiquitin-proteasome pathway and releasing NF- $\kappa$ B to enter the nucleus where it regulates gene expression (6-8). NIK and IKK $\alpha$  (IKK1) regulate the phosphorylation and processing of NF- $\kappa$ B2 (p100) to produce p52, which translocates to the nucleus (9-11).

## Background References

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- Thompson, J.E. et al. (1995) *Cell* 80, 573-82.
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- Traenckner, E.B. et al. (1995) *EMBO J* 14, 2876-83.
- Scherer, D.C. et al. (1995) *Proc Natl Acad Sci USA* 92, 11259-63.
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## Species Reactivity

Species reactivity is determined by testing in at least one approved application (e.g., western blot).

**Western Blot Buffer**

IMPORTANT: For western blots, incubate membrane with diluted primary antibody in 5% w/v nonfat dry milk, 1X TBS, 0.1% Tween® 20 at 4°C with gentle shaking, overnight.

**Applications Key**

**W:** Western Blotting **W-S:** Simple Western™ **IP:** Immunoprecipitation **IHC-P:** Immunohistochemistry (Paraffin) **IF-IC:** Immunofluorescence (Immunocytochemistry) **FC-FP:** Flow Cytometry (Fixed/Permeabilized) **ChIP:** Chromatin IP **ChIP-seq:** Chromatin IP-seq **C&R:** CUT&RUN

**Cross-Reactivity Key**

**H:** Human **M:** Mouse **R:** Rat **Hm:** Hamster **Mk:** Monkey **Dg:** Dog

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