Cell Signaling

✓ 100 µl (10 western blots)

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Applications W, IP, ChIP Endogenous Species Cross-Reactivity* H, M, (Mk) Molecular Wt. 50 kDa, Active form 120 kDa Precursor Isotype Rabbit IgG**

Background: Transcription factors of the nuclear factor κ B (NF-κB)/Rel family play a pivotal role in inflammatory and immune responses (1,2). There are five family members in mammals: ReIA, c-ReI, ReIB, NF- κ B1 (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- κ 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-κB to enter the nucleus where it regulates gene expression (6-8). NIK and IKK α (IKK1) regulate the phosphorylation and processing of NF-κB2 (p100) to produce p52, which translocates to the nucleus (9-11).

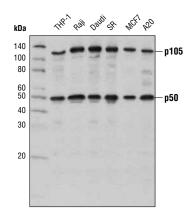
Following IKK-mediated phosphorylation of p105 NF- κ B at multiple sites (Ser921, 923, 927, and 932) on its carboxy-terminus, SCF/ β -TrCP mediated processing produces the 50 kDa active form p50 (12.13).

Specificity/Sensitivity: NF- κ B1 p105/p50 (D7H5M) Rabbit mAb recognizes endogenous levels of total NF- κ B1 p105/p50 protein.

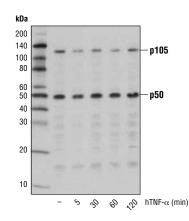
Source/Purification: Monoclonal antibody is produced by immunizing animals with a synthetic peptide corresponding to residues surrounding Gly415 of human NF- κ B p105/p50 protein.

Background References:

- (1) Baeuerle, P.A. and Henkel, T. (1994) *Annu Rev Immunol* 12, 141-79.
- (2) Baeuerle, P.A. and Baltimore, D. (1996) Cell 87, 13-20.
- (3) Haskill, S. et al. (1991) Cell 65, 1281-9.
- (4) Thompson, J.E. et al. (1995) Cell 80, 573-82.
- (5) Whiteside, S.T. et al. (1997) EMBO J 16, 1413-26.
- (6) Traenckner, E.B. et al. (1995) EMBO J 14, 2876-83.
- (7) Scherer, D.C. et al. (1995) Proc Natl Acad Sci USA 92, 11259-63.
- (8) Chen, Z.J. et al. (1996) Cell 84, 853-62.
- (9) Senftleben, U. et al. (2001) Science 293, 1495-9.
- (10) Coope, H.J. et al. (2002) EMBO J 21, 5375-85.



Western blot analysis of extracts from various cell lines using NF-κB1 p105/p50 (D7H5M) Rabbit mAb.



Western blot analysis of extracts from HeLa cells, untreated (-) or treated with Human Tumor Necrosis Factor-α. (hTNF-α) #8902 (10 ng/ml) for the indicated times, using NF-κB1 p105/p50 (D7H5M) Rabbit mAb.

- (11) Xiao, G. et al. (2001) Mol Cell 7, 401-9.
- (12) Heissmeyer, V. et al. (2001) Mol Cell Biol 21, 1024-35.
- (13) Orian, A. et al. (2000) EMBO J 19, 2580-91.

Entrez-Gene ID #4790 Swiss-Prot Acc. #P19838

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

*Species cross-reactivity is determined by western blot.

**Anti-rabbit secondary antibodies must be used to detect this antibody.

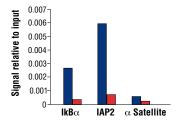
Recommended Antibody Dilutions:

Western blotting 1:1000
Immunoprecipitation 1:100
Chromatin IP 1:50

For product 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 complementary products.

■ NF-kB1 p105/p50 (D7H5M) Rabbit mAb #12540 ■ Normal Rabbit IgG #2729



Chromatin immunoprecipitations were performed with cross-linked chromatin from 4 x 10 $^{\circ}$ HeLa cells treated with Human Tumor Necrosis Factor- α (hTNF- α) #8902 (30 ng/ml) for 1 hour and either 10 μ l of NF- κ B1 p105/p50 (D7H5M) Rabbit mAb or 2 μ l of Normal Rabbit IgG #2729 using SimpleChIP $^{\circ}$ Enzymatic Chromatin IP Kit (Magnetic Beads) #9003. The enriched DNA was quantified by Real-Time PCR using Simple-ChIP $^{\circ}$ Human IkB α Promoter Primers #5552, human IAP2 promoter primers, and SimpleChIP $^{\circ}$ 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.

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

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