

#2697 Store at -20°C

Phospho-IKK α/β (Ser176/180) (16A6) Rabbit mAb

- Small 100 μl
(10 western blots)
- Large 300 μl
(30 western blots)



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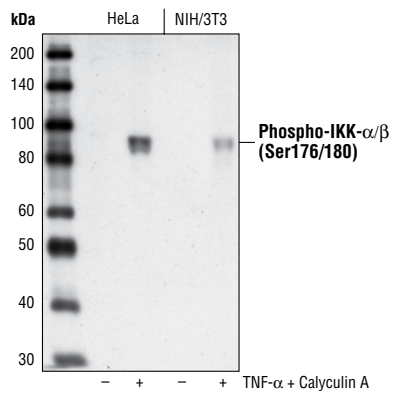
rev. 07/27/11

This product is intended for research purposes only. This product is not intended to be used for therapeutic or diagnostic purposes in humans or animals.

Entrez-Gene ID #1147
Swiss-Prot Acc. #O15111

Applications	Species Cross-Reactivity*	Molecular Wt.	Isotype
W, IHC-P, IHC-F Endogenous	H, M, R, Mk, (B)	85 kDa IKK- α 87 kDa IKK- β	Rabbit IgG**

Background: The NF κ B/Rel transcription factors are present in the cytosol in an inactive state, complexed with the inhibitory I κ B proteins (1–3). Most agents that activate NF κ B do so through a common pathway based on phosphorylation-induced, proteasome-mediated degradation of I κ B (3–7). The key regulatory step in this pathway involves activation of a high molecular weight I κ B kinase (IKK) complex, whose catalysis is generally carried out by three tightly associated IKK subunits. IKK α and IKK β serve as the catalytic subunits of the kinase. IKK γ serves as the regulatory subunit (8–9). Activation of IKK depends on phosphorylation; serines 177 and 181 in the activation loop of IKK β (176 and 180 in IKK α) are the specific sites whose phosphorylation causes conformational changes resulting in kinase activation (10–13).



Western blot analysis of extracts from TNF- α and calyculin A treated HeLa and NIH/3T3 cells, using Phospho-IKK- α/β (Ser176/180) (16A6) Rabbit mAb.

Specificity/Sensitivity: Phospho-IKK α/β (Ser176/180) (16A6) Rabbit mAb detects IKK α only when phosphorylated at Ser176/180 and IKK β only when phosphorylated at Ser177/181.

Source/Purification: Monoclonal antibody is produced by immunizing animals with a synthetic phosphopeptide corresponding to residues surrounding Ser176/180 of human IKK α .

Storage: Supplied in 10 mM sodium HEPES (pH 7.5), 150 mM NaCl, 100 $\mu\text{g}/\text{ml}$ 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
Immunohistochemistry (Paraffin)	1:150†
Unmasking buffer:	Citrate
Antibody diluent:	TBST-5%NGS
Detection reagent:	SignalStain [®] Boost (HRP, Rabbit) #8114
Immunohistochemistry (Frozen)	1:150†
Unmasking buffer:	Citrate
Antibody diluent:	TBST-5%NGS
Detection reagent:	SignalStain [®] Boost (HRP, Rabbit) #8114

†Optimal IHC dilutions determined using SignalStain[®] Boost IHC Detection Reagent.

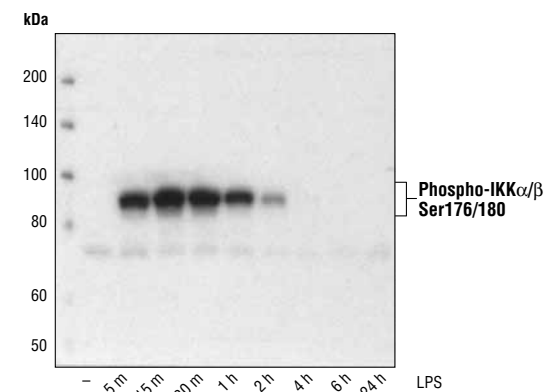
For application specific protocols please see the web page for this product at www.cellsignaling.com.

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

Background References:

- (1) Baeuerle, P.A. et al. (1988) *Science* 242, 540–546.
- (2) Beg, A.A. et al. (1993) *Genes Dev.* 7, 2064–2070.
- (3) Finco, T.S. et al. (1994) *Proc. Natl. Acad. Sci. USA* 91, 11884–11888.
- (4) Brown, K. et al. (1995) *Science* 267, 1485–1488.
- (5) Brockman, J.A. et al. (1995) *Mol. Cell. Biol.* 15, 2809–2818.
- (6) Traenckner, E.B. et al. (1995) *EMBO J.* 14, 2876–2883.
- (7) Chen, Z.J. et al. (1996) *Cell* 84, 853–862.
- (8) Zandi, E. et al. (1997) *Cell* 91, 243–252.
- (9) Karin, M. et al. (1999) *Oncogene* 18, 6867–6874.
- (10) DiDonato, J.A. et al. (1997) *Nature* 388, 548–554.
- (11) Mercurio, F. et al. (1997) *Science* 278, 860–866.
- (12) Johnson, L.N. et al. (1996) *Cell* 85, 149–158.
- (13) Delhase, M. et al. (1999) *Science* 284, 309–313.

Rabbit monoclonal antibody is produced under license (granting certain rights including those under U. S. Patents No. 5,675,063 and 7,429,487) from Epitomics, Inc.

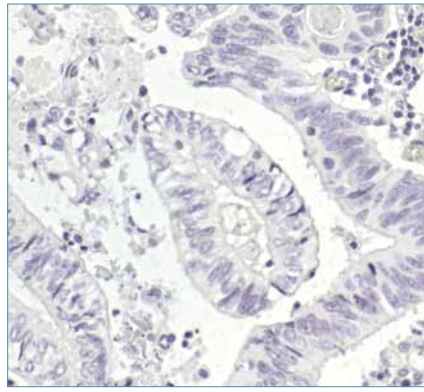
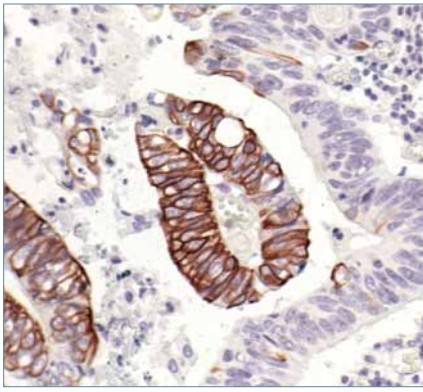


Western blot analysis of extracts from THP-1 cells, differentiated with TPA (#9905, 80 nM for 24h) and treated with 1 $\mu\text{g}/\text{ml}$ LPS for the indicated times, using Phospho-IKK α/β (Ser176/180) (16A6) Rabbit mAb.

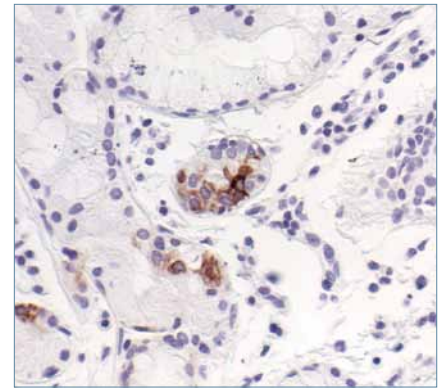
IMPORTANT: For western blots, incubate membrane with diluted antibody in 5% w/v BSA, 1X TBS, 0.1% Tween-20 at 4°C with gentle shaking, overnight.

Applications Key: W—Western IP—Immunoprecipitation IHC—Immunohistochemistry ChIP—Chromatin Immunoprecipitation IF—Immunofluorescence F—Flow cytometry E-P—ELISA-Peptide
Species Cross-Reactivity Key: H—human M—mouse R—rat Hm—hamster Mk—monkey Mi—mink C—chicken Dm—D. melanogaster X—Xenopus Z—zebrafish B—bovine
 Dg—dog Pg—pig Sc—S. cerevisiae Ce—C. elegans Hr—Horse All—all species expected Species enclosed in parentheses are predicted to react based on 100% homology.

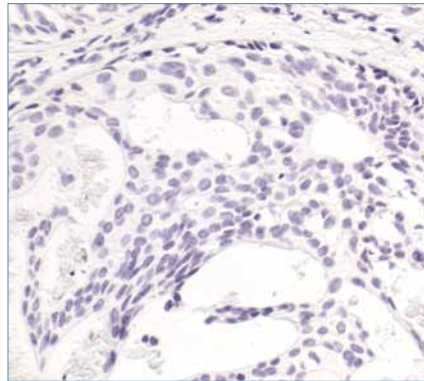
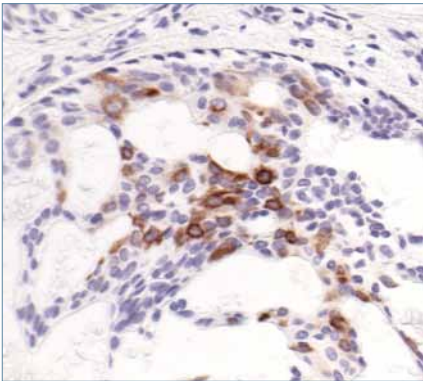
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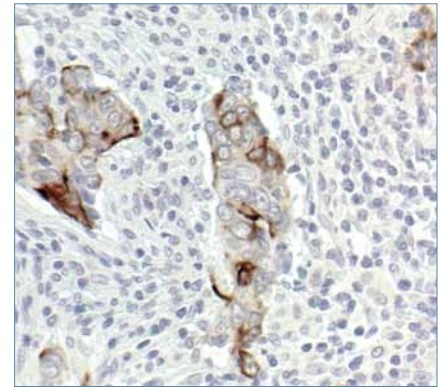
Immunohistochemical analysis of paraffin-embedded human colon carcinoma untreated (left) or λ -phosphatase-treated (right), using Phospho-IKK- α/β (Ser176/180) (16A6) Rabbit mAb.



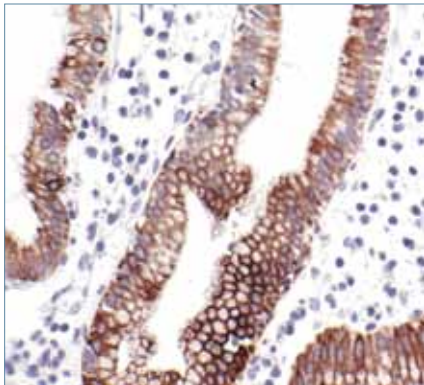
Immunohistochemical analysis of paraffin-embedded human lung (chronic bronchitis), using Phospho-IKK- α/β (Ser176/180) (16A6) Rabbit mAb.



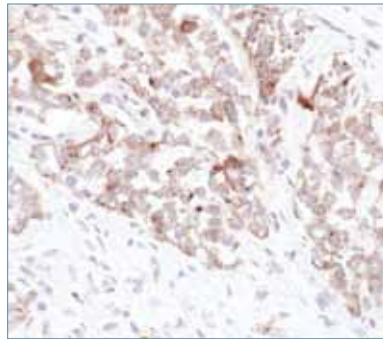
Immunohistochemical analysis of paraffin-embedded human breast carcinoma, using Phospho-IKK- α/β (Ser176/180) (16A6) Rabbit mAb in the presence of control peptide (left) or Phospho-IKK- α/β (Ser176/180) Blocking Peptide #1023 (right).



Immunohistochemical analysis of paraffin-embedded human colon carcinoma, showing cytoplasmic localization, using Phospho-IKK- α/β (Ser176/180) (16A6) Rabbit mAb.



Immunohistochemical analysis of paraffin-embedded human gall bladder (chronic cholecystitis), using Phospho-IKK- α/β (Ser176/180) (16A6) Rabbit mAb.



Immunohistochemical analysis of frozen H1650 xenograft, showing cytoplasmic localization using Phospho-IKK- α/β (Ser176/180) (16A6) Rabbit mAb.