Store at -20°C

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

#2697

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Entrez-Gene ID #1147
UniProt ID #O15111

rev. 12/29/15

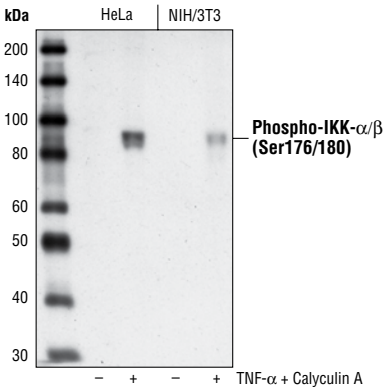
For Research Use Only. Not For Use In Diagnostic Procedures.

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

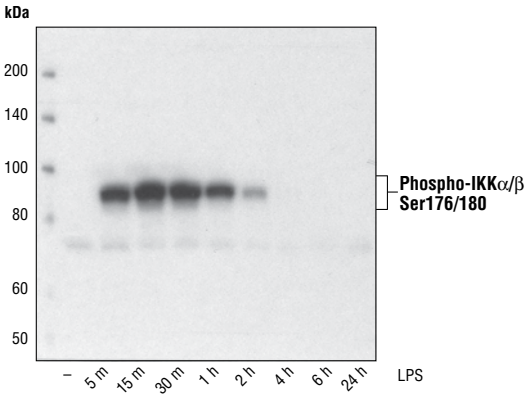
Background: The NF-κB/Rel transcription factors are present in the cytosol in an inactive state, complexed with the inhibitory IκB proteins (1-3). Most agents that activate NF-κB do so through a common pathway based on phosphorylation-induced, proteasome-mediated degradation of IκB (3-7). The key regulatory step in this pathway involves activation of a high molecular weight IκB kinase (IKK) complex whose catalysis is generally carried out by three tightly associated IKK subunits. IKKα and IKKβ serve as the catalytic subunits of the kinase and IKKγ serves as the regulatory subunit (8,9). Activation of IKK depends upon phosphorylation at Ser177 and Ser181 in the activation loop of IKKβ (Ser176 and Ser180 in IKKα), which causes conformational changes, resulting in kinase activation (10-13).

Specificity/Sensitivity: Phospho-IKKα/β (Ser176/180) (16A6) Rabbit mAb detects IKKα only when phosphorylated at Ser176/180 and IKKβ 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 IKKα.



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.



Western blot analysis of extracts from THP-1 cells, differentiated with TPA (#9905, 80 nM for 24h) and treated with 1 µg/ml LPS for the indicated times, using Phospho-IKKα/β (Ser176/180) (16A6) Rabbit mAb.

Storage: Supplied in 10 mM sodium HEPES (pH 7.5), 150 mM NaCl, 100 µg/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.

Flow Cytometry	1:3200

For product specific protocols and a complete listing of recommended companion products please see the product web page at www.cellsignal.com

Background References:

(1) Baeuerle, P.A. and Baltimore, D. (1988) *Science* 242, 540-6.

(2) Beg, A.A. and Baldwin, A.S. (1993) *Genes Dev* 7, 2064-70.

(3) Finco, T.S. et al. (1994) *Proc Natl Acad Sci USA* 91, 11884-8.

(4) Brown, K. et al. (1995) *Science* 267, 1485-8.

(5) Brockman, J.A. et al. (1995) *Mol Cell Biol* 15, 2809-18.

(6) Traenckner, E.B. et al. (1995) *EMBO J* 14, 2876-83.

(7) Chen, Z.J. et al. (1996) *Cell* 84, 853-62.

(8) Zandi, E. et al. (1997) *Cell* 91, 243-52.

(9) Karin, M. (1999) *Oncogene* 18, 6867-74.

(10) DiDonato, J.A. et al. (1997) *Nature* 388, 548-54.

(11) Mercurio, F. et al. (1997) *Science* 278, 860-6.

(12) Johnson, L.N. et al. (1996) *Cell* 85, 149-58.

(13) Delhase, M. et al. (1999) *Science* 284, 309-13.

U. S. Patent No. 5,675,063
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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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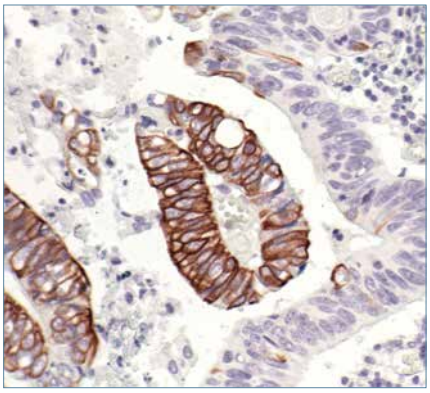
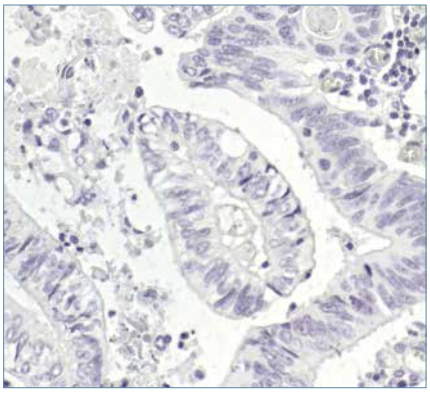
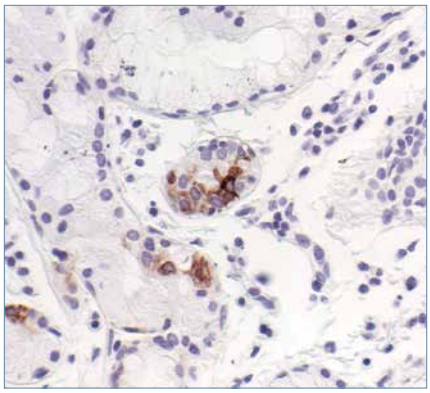
Applications: **W**—Western **IP**—Immunoprecipitation **IHC**—Immunohistochemistry **ChIP**—Chromatin Immunoprecipitation **IF**—Immunofluorescence **F**—Flow cytometry **E-P**—ELISA-Peptide **Species Cross-Reactivity:** **H**—human **M**—mouse **R**—rat **Hm**—hamster **Mk**—monkey **Mi**—mink **C**—chicken **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.

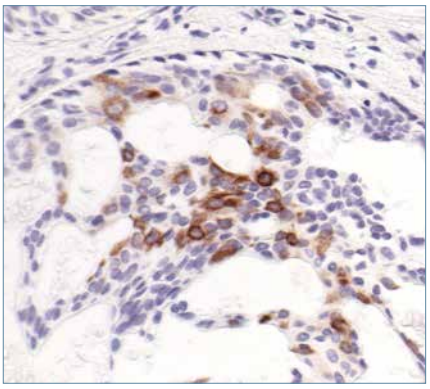


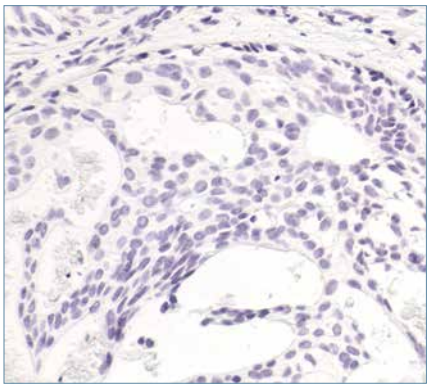


Immunohistochemical analysis of paraffin-embedded human colon carcinoma untreated (left) or λ-phosphatase-treated (right), using Phospho-IKK-α/β (Ser176/180) (16A6) Rabbit mAb.

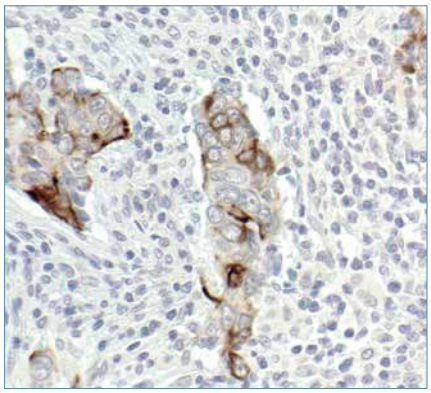


Immunohistochemical analysis of paraffin-embedded human lung (chronic bronchitis), using Phospho-IKK-α/β (Ser176/180) (16A6) Rabbit mAb.

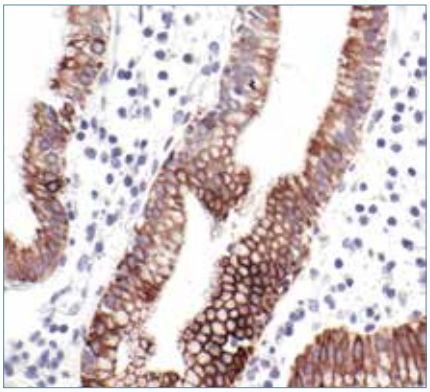




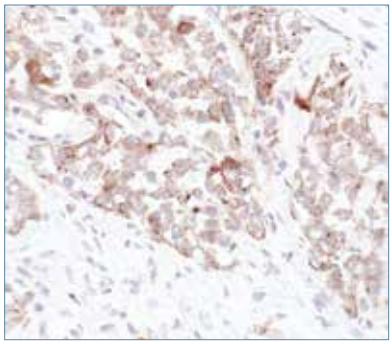
Immunohistochemical analysis of paraffin-embedded human breast carcinoma, using Phospho-IKK-α/β (Ser176/180) (16A6) Rabbit mAb in the presence of control peptide (left) or Phospho-IKK-α/β (Ser176/180) Blocking Peptide #1023 (right).



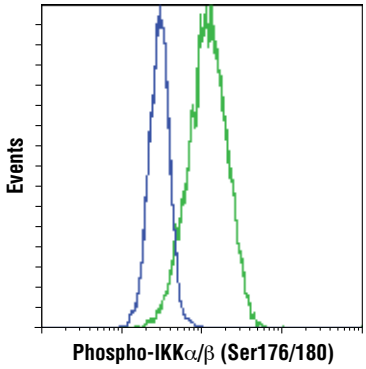
Immunohistochemical analysis of paraffin-embedded human colon carcinoma, showing cytoplasmic localization, using Phospho-IKK-/ (Ser176/180) (16A6) Rabbit mAb.



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.



Flow cytometric analysis of THP-1 cells, untreated (blue) and with TPA and LPS (green) using IKK-α (Ser176/Ser180) phosphate Rabbit mAb. Anti-rabbit IgG (H+L), F(ab')$_2$ Fragment (PE Conjugate) #8885 was used as a secondary antibody

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