Store at –20°C

#9240

PhosphoPlus® IκBα (Ser32/36) Antibody Kit

☑ 10 western blots per primary antibody

Cell Signaling TECHNOLOGY®

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orders@cellsignal.com
Support ■ 877-678-TECH (8324)
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For Research Use Only. Not For Use In Diagnostic Procedures.

Products Included	Product #	Quantity	Mol. Wt.	Isotype
IκB-α (L35A5) Mouse mAb (Amino-terminal Antigen)	4814	100 µl	39 kDa	Mouse IgG1
Phospho-IκB-α (Ser32/36) (5A5) Mouse mAb	9246	100 µl	40 kDa	Mouse IgG1
NF-κB Control Cell Extracts	9243	200 µl		
Anti-mouse IgG, HRP-linked Antibody	7076	100 µl		Horse
Anti-biotin, HRP-linked Antibody	7075	100 µl		Goat
20X LumiGLO® Reagent and 20X Peroxide	7003	5 ml each		
Biotinylated Protein Ladder	7727	100 µl		

See www.cellsignal.com for individual component applications, species cross-reactivity, dilutions and additional application protocols.

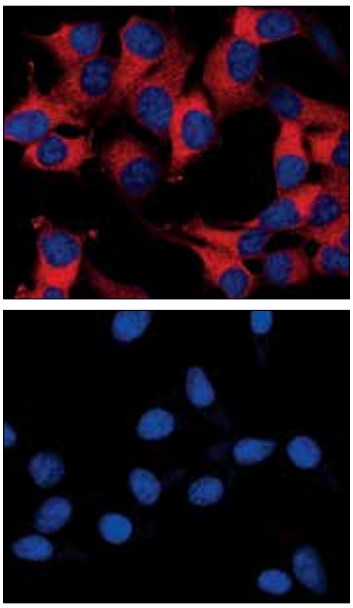
Description: The PhosphoPlus® IκBα (Ser32/36) Antibody Kit provides reagents and protocols for the rapid analysis of IκBα phosphorylation at Ser32/36. The kit contains a total IκBα antibody, a phospho-specific antibody, and positive/negative control whole cell lysates, along with secondary antibodies and reagents for Western blotting.

Background: The NF-kappaB/Rel transcription factors are present in the cytosol in an inactive state complexed with the inhibitory IkappaB proteins (1-3). Activation occurs via phosphorylation of IkappaB-alpha at Ser32 and Ser36 followed by proteasome-mediated degradation, resulting in the release and nuclear translocation of active NF-kappaB (3-7). IkappaB-alpha phosphorylation and resulting Rel-dependent transcription are activated by a highly diverse group of extracellular signals including inflammatory cytokines, growth factors and chemokines.

Kinases that phosphorylate IkappaB at these activating sites have been identified (8). Because phosphorylation of IkappaB-alpha at Ser32/36 is essential for release of active NF-kappaB, phosphorylation at this site is an excellent marker of NF-kappaB activation (1-3).

Specificity/Sensitivity: IκBα (L35A5) Mouse mAb (Amino-terminal Antigen) #4814 detects endogenous levels of total IκBα protein. Phospho-IκBα (Ser32/36) (5A5) Mouse mAb #9246 detects endogenous levels of IκBα only when phosphorylated at Ser32/36. Neither antibody cross-reacts with other IκB family members at physiological levels.

Source/Purification: IκBα (L35A5) Mouse mAb (Amino-terminal Antigen) #4814 is produced by immunizing mice with a GST-IκBα fusion protein corresponding the amino-terminus of human IκBα. Phospho-IκBα (Ser32/36) (5A5) Mouse mAb #9246 is produced by immunizing mice with a synthetic phospho-peptide (KLH-coupled) corresponding to residues surrounding Ser32/36 of human IκBα. Total cell extracts from HeLa cells prepared without treatment serve as a negative control. Supplied in SDS Sample Buffer. Total cell extracts from HeLa cells prepared with TNFα treatment (#2169, 20 ng/ml for 5 minutes) serve as a positive control. Supplied in SDS Sample Buffer.



Confocal immunofluorescent analysis of HeLa cells, untreated (upper), or TNFα-treated (lower) using ***IκB-α (L35A5) Mouse mAb (Amino-terminal Antigen) #4814*** *(red). Blue pseudocolor = DRAQ5® (fluorescent DNA dye).*

Storage: Antibodies are supplied in 10 mM sodium HEPES (pH 7.5), 150 mM NaCl, 100 µg/ml BSA and 50% glycerol. Store at –20°C. Do not aliquot the antibodies. NF-κB Control Cell Extracts are supplied in SDS Sample Buffer: 62.5 mM Tris-HCl (pH 6.8 at 25°C), 2% w/v SDS, 10% glycerol, 50 mM DTT, 0.01% w/v bromophenol blue or phenol red. Store at –20°C or below.

Recommended Antibody Dilutions:

Western blotting	1:1000

See www.cellsignal.com for individual component dilutions

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

DRAQ5 is a registered trademark of Biostatus Limited.
LumiGLO is a registered trademark of Kirkegaard & Perry Laboratories.

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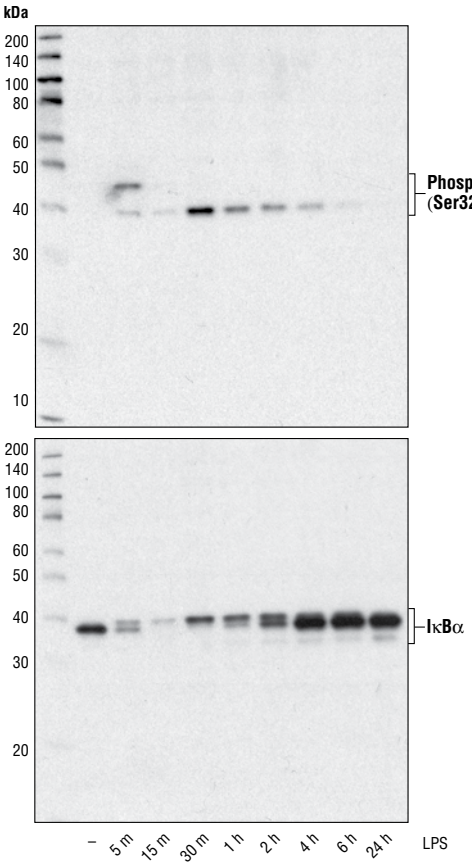
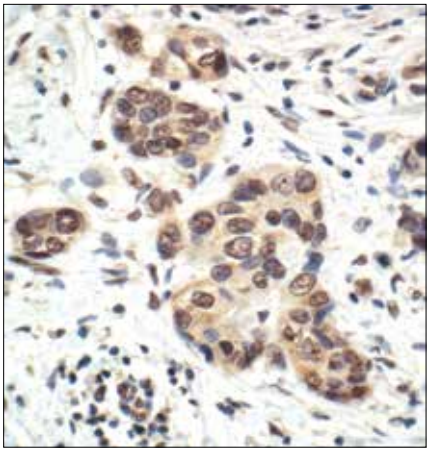
Applications Key: **W**—Western **IP**—Immunoprecipitation **IHC**—Immunohistochemistry **ChIP**—Chromatin Immunoprecipitation **IF**—Immunofluorescence **F**—Flow cytometry **E-P**—ELISA-Peptide

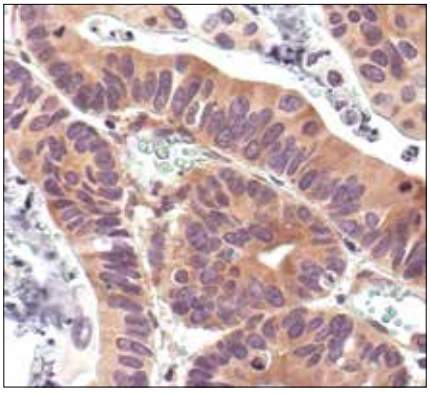
Species Cross-Reactivity Key: **H**—human **M**—mouse **R**—rat **Hm**—hamster **Mk**—monkey **Mi**—mink **C**—chicken **Dm**—D. melanogaster **X**—Xenopus **Z**—zebrafish **B**—bovine **Dg**—dog **Pg**—pig **Sc**—S. cerevisiae **All**—all species expected Species enclosed in parentheses are predicted to react based on 100% homology.



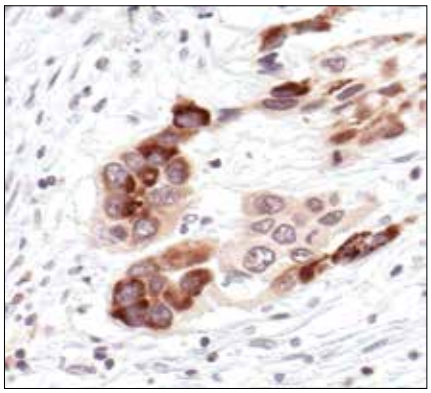
Western blot analysis of extracts from THP-1 cells, differentiated with TPA (#9905, 80 nM for 24h) and treated with 1 µg/ml LPS for the indicated times, using ***Phospho-IκBα (Ser32/36) (5A5) Mouse mAb #9246*** *(upper) and* ***IκBα (L35A5) Mouse mAb (Amino-terminal Antigen) #4814*** *(lower).*



Immunohistochemical analysis of paraffin-embedded human breast tumor, using ***Phospho-IκB-α (Ser32/36) (5A5) Mouse mAb #9246****.*



Immunohistochemical analysis of paraffin-embedded human renal adenocarcinoma, using ***IκB-α (L35A5) Mouse mAb (Amino-terminal Antigen) #4814****.*



Immunohistochemical analysis of paraffin-embedded human lung carcinoma, using ***IκB-α (L35A5) Mouse mAb (Amino-terminal Antigen) #4814****.*

Background References:

(1) Baeuerle, P.A. and Baltimore, D. (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) Karin, M. and Ben-Neriah, Y. (2000) *Annu. Rev. Immunol.* 18, 621–663.

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Western Immunoblotting Protocol (Primary Antibody Incubation in Milk)

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

A Solutions and Reagents

NOTE: Prepare solutions with Milli-Q or equivalently purified water.

1. 1X Phosphate Buffered Saline (PBS)
2. **1X SDS Sample Buffer:** 62.5 mM Tris-HCl (pH 6.8 at 25°C), 2% w/v SDS, 10% glycerol, 50 mM DTT, 0.01% w/v bromophenol blue or phenol red
3. **Transfer Buffer:** 25 mM Tris base, 0.2 M glycine, 20% methanol (pH 8.5)
4. **10X Tris Buffered Saline (TBS):** To prepare 1 liter of 10X TBS: 24.2 g Tris base, 80 g NaCl; adjust pH to 7.6 with HCl (use at 1X).
5. Nonfat Dry Milk (weight to volume [w/v])
6. **Blocking Buffer:** 1X TBS, 0.1% Tween®20 with 5% w/v nonfat dry milk; for 150 ml, add 15 ml 10X TBS to 135 ml water, mix. Add 7.5 g nonfat dry milk and mix well. While stirring, add 0.15 ml Tween®20 (100%).
7. **Wash Buffer:** 1X TBS, 0.1% Tween®20 (TBS/T)
8. **Primary Antibody Dilution Buffer:** 1X TBS, 0.1% Tween®20 with 5% nonfat dry milk; for 20 ml, add 2 ml 10X TBS to 18 ml water, mix. Add 1.0 g nonfat dry milk and mix well. While stirring, add 20 µl Tween®20 (100%).
9. **Phototope®-HRP Western Blot Detection System** #7072**:** Includes biotinylated protein ladder, secondary anti-mouse (#7076) antibody conjugated to horseradish peroxidase (HRP), anti-biotin antibody conjugated to HRP, LumiGLO® chemiluminescent reagent and peroxide.
10. Prestained Protein Marker, Broad Range (Premixed Format) #7720
11. Biotinylated Protein Ladder Detection Pack #7727
12. **Blotting Membrane:** This protocol has been optimized for nitrocellulose membranes, which CST recommends. PVDF membranes may also be used.

B Protein Blotting

A general protocol for sample preparation is described below.

1. Treat cells by adding fresh media containing regulator for desired time.
2. Aspirate media from cultures; wash cells with 1X PBS; aspirate.
3. Lyse cells by adding 1X SDS sample buffer (100 µl per well of 6-well plate or 500 µl per plate of 10 cm diameter plate). Immediately scrape the cells off the plate and transfer the extract to a microcentrifuge tube. Keep on ice.
4. Sonicate for 10–15 seconds to shear DNA and reduce sample viscosity.
5. Heat a 20 µl sample to 95–100°C for 5 minutes; cool on ice.
6. Microcentrifuge for 5 minutes.
7. Load 20 µl onto SDS-PAGE gel (10 cm x 10 cm).

NOTE: CST recommends loading prestained molecular weight marker (#7720, 10 µl/lane) to verify electrotransfer and biotinylated protein ladder (#7727, 10 µl/lane) to determine molecular weights.

8. Electrotransfer to nitrocellulose or PVDF membrane.

C Membrane Blocking and Antibody Incubations

NOTE: Volumes are for 10 cm x 10 cm (100 cm^2) of membrane; for different sized membranes, adjust volumes accordingly.

1. (Optional) After transfer, wash nitrocellulose membrane with 25 ml TBS for 5 minutes at room temperature.
2. Incubate membrane in 25 ml of blocking buffer for 1 hour at room temperature.
3. Wash three times for 5 minutes each with 15 ml of TBS/T.
4. Incubate membrane and primary antibody (at the appropriate dilution) in 10 ml primary antibody dilution buffer with gentle agitation overnight at 4°C.
5. Wash three times for 5 minutes each with 15 ml of TBS/T.
6. Incubate membrane with HRP-conjugated secondary antibody (1:2000) and HRP-conjugated anti-biotin antibody (1:1000) to detect biotinylated protein markers in 10 ml of blocking buffer with gentle agitation for 1 hour at room temperature.
7. Wash three times for 5 minutes each with 15 ml of TBS/T.

D Detection of Proteins

1. Incubate membrane with 10 ml LumiGLO® (0.5 ml 20X LumiGLO®, 0.5 ml 20X Peroxide and 9.0 ml Milli-Q water) with gentle agitation for 1 minute at room temperature.

NOTE: LumiGLO® substrate can be further diluted if signal response is too fast.

2. Drain membrane of excess developing solution (do not let dry), wrap in plastic wrap and expose to x-ray film. An initial 10-second exposure should indicate the proper exposure time.

NOTE: Due to the kinetics of the detection reaction, signal is most intense immediately following LumiGLO® incubation and declines over the following 2 hours.

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MSDS

Material Safety Data Sheet (MSDS) for Antibodies

Cell Signaling TECHNOLOGY®

rev. 08/09/07

I. Identification:

Product name: Antibodies
Product Catalog Number: Includes antibodies within the following range of catalog numbers: 2000-5999, 7000-7999 and 9000-9999.
CAS number: None

Manufacturer Supplier: Cell Signaling Technology
3 Trask Lane
Danvers, MA 01923 USA
1-978-867-2300 TEL
1-978-867-2400 FAX
1-978-578-6737 Emergency Phone

II. Composition/Information on Ingredients:

This product is composed of antibodies in aqueous buffer solution. According to 29 CFR 1910.1200(d), hazardous ingredients at less than <1% and carcinogens at less than < 0.1% are considered non-hazardous. Any hazardous or carcinogenic ingredients exceeding these criteria are listed below.

This product may contain the following hazardous ingredients.

Ingredient	CAS#	Percent
Glycerol	56-81-5	50%

III. Hazard Identification:

Emergency Overview of Hazardous ingredient: Glycerol (CAS# 56-81-5)
Caution: Avoid contact and inhalation.
Target Organ: Kidneys.

NFPA Rating:

Health Rating:	1
Flammability Rating:	0
Reactivity Rating:	0

IV. First Aid Measures:

Inhalation: If inhaled, remove to fresh air. If breathing is difficult, get medical attention.
Ingestion: If swallowed and person is conscious, rinse out mouth with water. Get medical attention.
Skin Exposure: In case of contact, wash skin with soap and water.
Eye Exposure: In case of contact with eyes, immediately flush eyes water for at least 15 minutes. Get medical attention.

V. Fire Fighting Measures:

Flash Point: Data not available.
Autoignition Temperature: Data not available.
Fire Extinguishing Media: Water spray, dry chemical, foam, or carbon dioxide.
Firefighting: Wear protective clothing and self-contained breathing apparatus to prevent contact with skin and eyes.

VI. Accidental Release Measures:

Absorb liquid with an absorbent material. Transfer contaminated absorbent to a chemical waste container for disposal.

VII. Handling And Storage:

Avoid inhalation and contact with eyes and skin. Avoid prolonged or repeated exposure. Store at –20°C in tightly closed container.

VIII. Exposure Controls/Personal

Engineering Controls: Maintain adequate ventilation, eye wash and quick-drench facilities in work area.
Personal Protective Equipment: Lab coat, chemical resistant gloves and chemical safety glasses.
Occupational Exposure Limits: Data not available.

IX. Exposure Controls/Personal Protection:

Physical State:	Colorless liquid.
Odor:	Odorless.
Boiling Point:	Data not available.
Melting Point:	Data not available.
Volatile Organic Compound:	Data not available.
Solubility in water:	Readily miscible in water.

X. Stability and Reactivity:

Stability: Stable.
Hazardous Decomposition: May form carbon dioxide and carbon monoxide.
Conditions to avoid: Strong oxidizing agents

XI. Toxicological Information:

May cause skin irritation.
May be toxic if absorbed through skin or ingested.
May cause eye irritation.

Target Organs: Kidneys
Prolonged exposure may cause nausea, headache, and vomiting.

XII. Ecological Information:

Data not available.

XIII. Disposal Considerations:

Dispose of in accordance with federal, state and local environmental regulations.

XIV. Transport Information:

D.O.T.: This substance is considered non-hazardous for transport.
IATA: This substance is considered non-hazardous for air transport.

XV. Regulatory Information:

EU Regulation/Classification/Labeling Information: Not available for this product.

Chemical Inventory Status:
SARA Listed Component: None.
TSCA Listed Component: None.
Canada (WHMIS): DSL No, NDSL No.

XVI. Other Information:

This compound is sold only for research use by personnel familiar with chemicals and who are well trained in good laboratory habits, such as avoiding spills, keeping hands clean at all times and not rubbing eyes with hands while working in the laboratory.

This solution is sold only in microliter quantities for use in life sciences research. No other use is intended, and any other use may involve substantive hazards.

The above information is believed to be correct but does not purport to be all inclusive and shall be used only as a guide for experienced personnel. Cell Signaling Technology, Inc., shall not be held liable for any damage resulting from the handling of or from contact with the above product. The burden of safe use of this material rests entirely with the user.

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