

PhosphoPlus® p44/42 MAPK (Erk1/2) (Thr202/Tyr204) Antibody Kit

1 Kit
 (20 western blots)



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rev. 03/31/16

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

Products Included	Product #	Quantity	Mol. Wt.	Isotype
Phospho-p44/42 MAPK (Erk1/2) (Thr202/Tyr204) (D13.14.4E) XP® Rabbit mAb	4370	200 µl	42, 44 kDa	Rabbit IgG
p44/42 MAPK (Erk1/2) (137F5) Rabbit mAb	4695	200 µl	42, 44 kDa	Rabbit IgG
Anti-rabbit IgG, HRP-linked Antibody	7074	100 µl		Goat
p44/42 MAPK (Erk1/2) Control Cell Extracts	9194	150 µl	42, 44 kDa	
Anti-biotin, HRP-linked Antibody	7075	250 µl		Goat
Biotinylated Protein Ladder	7727	250 µl		
20X LumiGLO® Reagent and 20X Peroxide	7003	10 ml each		

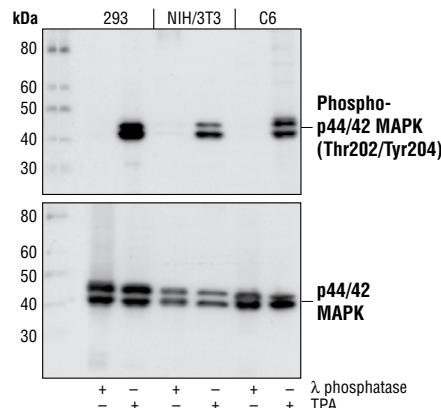
See www.cellsignal.com for individual component applications, species cross-reactivity, dilutions and additional application protocols.

Description: The PhosphoPlus® p44/42 MAPK (Erk1/2) (Thr202/Tyr204) Antibody Kit provides reagents and protocols for the rapid analysis of the phosphorylation status of p44 and p42 (Erk1 and Erk2). p44/42 MAPK (Erk1/2) p44/42 MAPK (Erk1/2) Negative Control Cell Extracts: Total cell extracts from Jurkat cells treated with U0126 (MEK1/2 Inhibitor) #9903 at 10 µM for 1 hour, to serve as a negative control. Supplied in SDS Sample Buffer. p44/42 MAPK (Erk1/2) Positive Control Cell Extracts: Total cell extracts from Jurkat cells treated with TPA #4174 at 200 nM for 20 minutes, to serve as a positive control. Supplied in SDS Sample Buffer.

Background: Mitogen-activated protein kinases (MAPKs) are a widely conserved family of serine/threonine protein kinases involved in many cellular programs such as cell proliferation, differentiation, motility, and death. The p44/42 MAPK (ERK1/2) signaling pathway can be activated in response to a diverse range of extracellular stimuli including mitogens, growth factors, and cytokines (1-3) and is an important target in the diagnosis and treatment of cancer (4). Upon stimulation, a sequential three-part protein kinase cascade is initiated, consisting of a MAP kinase kinase kinase (MAPKKK or MAP3K), a MAP kinase kinase (MAPKK or MAP2K), and a MAP kinase (MAPK). Multiple p44/42 MAP3Ks have been identified, including members of the Raf family as well as Mos and Tpl2/Cot. MEK1 and MEK2 are the primary MAPKKs in this pathway (5,6). MEK1 and MEK2 activate p44 and p42 through phosphorylation of activation loop residues Thr202/Tyr204 and Thr185/Tyr187, respectively. Several downstream targets of p44/42 have been identified, including p90RSK (7) and the transcription factor Elk-1 (8,9). p44/42 are negatively regulated by a family of dual-specificity (Thr/Tyr) MAPK phosphatases, known as DUSPs or MKPs (10), along with MEK inhibitors such as U0126 and PD98059.

Specificity/Sensitivity: Phospho-p44/42 MAPK (Erk1/2) (Thr202/Tyr204) (D13.14.4E) XP® Rabbit mAb #4370 detects endogenous levels of p42 and p44 MAP Kinase (Erk1 and Erk2) when dually phosphorylated at Thr202 and Tyr204 of Erk1 (Thr185 and Tyr187 of Erk2), and singly phosphorylated at Thr202. This antibody does not cross-react with the corresponding phosphorylated residues of either JNK/SAPK or p38 MAP kinases. p44/42 MAPK (Erk1/2) (137F5) Rabbit mAb #4695 detects endogenous levels of total p44/42 MAP kinase (Erk1/Erk2) protein. The antibody does not cross-react with either JNK/SAPK or p38 MAP kinases.

Source/Purification: Monoclonal antibody is produced by immunizing animals with a synthetic phosphopeptide corresponding to residues surrounding Thr202/Tyr204 of human p44 MAP kinase or a synthetic peptide corresponding to residues near the C-terminus of rat p44 MAP kinase.



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.

p44/42 MAPK (Erk1/2) 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 -80°C for long-term storage.

Recommended Antibody Dilutions:
 Western blotting 1:1000 (#4695)
 Western blotting 1:2000 (#4370)

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

Background References:

- (1) Roux, P.P. and Blenis, J. (2004) *Microbiol Mol Biol Rev* 68, 320–44.
- (2) Baccharini, M. (2005) *FEBS Lett* 579, 3271–7.
- (3) Meloche, S. and Pouyssegur, J. (2007) *Oncogene* 26, 3227–39.
- (4) Roberts, P.J. and Der, C.J. (2007) *Oncogene* 26, 3291–310.
- (5) Rubinfeld, H. and Seger, R. (2005) *Mol Biotechnol* 31, 151–74.
- (6) Murphy, L.O. and Blenis, J. (2006) *Trends Biochem Sci* 31, 268–75.
- (7) Dalby, K.N. et al. (1998) *J Biol Chem* 273, 1496–505.
- (8) Marais, R. et al. (1993) *Cell* 73, 381–93.
- (9) Kortenjann, M. et al. (1994) *Mol Cell Biol* 14, 4815–24.
- (10) Owens, D.M. and Keyse, S.M. (2007) *Oncogene* 26, 3203–13.

◀ Western blot analysis of extracts from 293, NIH/3T3 and C6 cells, treated with λ phosphatase or TPA as indicated, using Phospho-p44/42 MAPK (Erk1/2) (Thr202/Tyr204) (D13.14.4E) XP® Rabbit mAb #4370 (upper), or p44/42 MAPK (Erk1/2) (137F5) Rabbit mAb #4695 (lower).

U.S. Patent No. 5,675,063

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.

Western Immunoblotting Protocol

For western blots, incubate membrane with diluted primary antibody in either 5% w/v BSA or nonfat dry milk, 1X TBS, 0.1% Tween® 20 at 4°C with gentle shaking, overnight.
NOTE: Please refer to primary antibody datasheet or product webpage for recommended primary antibody dilution buffer and recommended antibody dilution.

A. Solutions and Reagents

NOTE: Prepare solutions with reverse osmosis deionized (RODI) or equivalent grade water.

- 20X Phosphate Buffered Saline (PBS):** (#9808) To prepare 1 L 1X PBS: add 50 ml 20X PBS to 950 ml dH₂O, mix.
- 10X Tris Buffered Saline (TBS):** (#12498) To prepare 1 L 1X TBS: add 100 ml 10X to 900 ml dH₂O, mix.
- 1X SDS Sample Buffer:** Blue Loading Pack (#7722) or Red Loading Pack (#7723)
Prepare fresh 3X reducing loading buffer by adding 1/10 volume 30X DTT to 1 volume of 3X SDS loading buffer. Dilute to 1X with dH₂O.
- 10X Tris-Glycine SDS Running Buffer:** (#4050) To prepare 1 L 1X running buffer: add 100 ml 10X running buffer to 900 ml dH₂O, mix.
- 10X Tris-Glycine Transfer Buffer:** (#12539) To prepare 1 L 1X transfer buffer: add 100 ml 10X transfer buffer to 200 ml methanol + 700 ml dH₂O, mix.
- 10X Tris Buffered Saline with Tween® 20 (TBST):** (#9997) To prepare 1 L 1X TBST: add 100 ml 10X TBST to 900 ml dH₂O, mix.
- Nonfat Dry Milk:** (#9999)
- Blocking Buffer:** 1X TBST with 5% w/v nonfat dry milk; for 150 ml, add 7.5 g nonfat dry milk to 150 ml 1X TBST and mix well.
- Wash Buffer:** (#9997) 1X TBST
- Bovine Serum Albumin (BSA):** (#9998)
- Primary Antibody Dilution Buffer:** 1X TBST with 5% BSA or 5% nonfat dry milk as indicated on primary antibody datasheet; for 20 ml, add 1.0 g BSA or nonfat dry milk to 20 ml 1X TBST and mix well.
- Biotinylated Protein Ladder Detection Pack:** (#7727)
- Prestained Protein Marker, Broad Range (Premixed Format):** (#7720)
- Blotting Membrane and Paper:** (#12369) This protocol has been optimized for nitrocellulose membranes. Pore size 0.2 µm is generally recommended.
- Secondary Antibody Conjugated to HRP:** anti-rabbit (#7074); anti-mouse (#7076)
- Detection Reagent:** LumiGLO® chemiluminescent reagent and peroxide (#7003) or SignalFire™ ECL Reagent (#6883)

B. Protein Blotting

A general protocol for sample preparation.

- Treat cells by adding fresh media containing regulator for desired time.
- Aspirate media from cultures; wash cells with 1X PBS; aspirate.
- Lyse cells by adding 1X SDS sample buffer (100 µl per well of 6-well plate or 500 µl for a 10 cm diameter plate). Immediately scrape the cells off the plate and transfer the extract to a microcentrifuge tube. Keep on ice.
- Sonicate for 10–15 sec to complete cell lysis and shear DNA (to reduce sample viscosity).
- Heat a 20 µl sample to 95–100°C for 5 min; cool on ice.
- Microcentrifuge for 5 min.
- Load 20 µl onto SDS-PAGE gel (10 cm x 10 cm). **NOTE:** Loading of prestained molecular weight markers (#7720, 10 µl/lane) to verify electrotransfer and biotinylated protein ladder (#7727, 10 µl/lane) to determine molecular weights are recommended.
- Electrotransfer to nitrocellulose membrane (#12369).

C. Membrane Blocking and Antibody Incubations

NOTE: Volumes are for 10 cm x 10 cm (100 cm²) of membrane; for different sized membranes, adjust volumes accordingly.

I. Membrane Blocking

- (Optional) After transfer, wash nitrocellulose membrane with 25 ml TBS for 5 min at room temperature.
- Incubate membrane in 25 ml of blocking buffer for 1 hr at room temperature.
- Wash three times for 5 min each with 15 ml of TBST.

II. Primary Antibody Incubation

- Incubate membrane and primary antibody (at the appropriate dilution and diluent as recommended in the product datasheet) in 10 ml primary antibody dilution buffer with gentle agitation overnight at 4°C.
- Wash three times for 5 min each with 15 ml of TBST.
- Incubate membrane with the species appropriate HRP-conjugated secondary antibody (#7074 or #7076 at 1:2000) and anti-biotin, HRP-linked Antibody (#7075 at 1:1000–1:3000) to detect biotinylated protein markers in 10 ml of blocking buffer with gentle agitation for 1 hr at room temperature.
- Wash three times for 5 min each with 15 ml of TBST.
- Proceed with detection (Section D).

D. Detection of Proteins

- Incubate membrane with 10 ml LumiGLO® (0.5 ml 20X LumiGLO® #7003, 0.5 ml 20X peroxide, and 9.0 ml purified water) or 10 ml SignalFire™ #6883 (5 ml Reagent A, 5 ml Reagent B) with gentle agitation for 1 min at room temperature.
- Drain membrane of excess developing solution (do not let dry), wrap in plastic wrap and expose to x-ray film. An initial 10 sec exposure should indicate the proper exposure time.
NOTE: Due to the kinetics of the detection reaction, signal is most intense immediately following incubation and declines over the following 2 hr.

LumiGLO® is a registered trademark of Kirkegaard & Perry Laboratories. Tween® is a registered trademark of ICI Americas, INC. SignalFire™ is a trademark of Cell Signaling Technology, INC.

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Material Safety Data Sheet (MSDS) for 20X LumiGLO® and 20X Peroxide



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Product name: 20X LumiGLO® and 20X Peroxide

Product Catalog: 7003

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 TEL

II. Composition/Information on Ingredients:

Hazardous Reagent:	Percent	CAS#
Dimethyl sulfoxide	≤20%	67-68-5

This product is For Research Use Only. According to 29 CFR 1910.1200(d), mixtures with hazardous ingredients at less than <1% and carcinogens at less than <0.1% are considered non-hazardous.

III. Hazard Identification:

CAUTION: This product is not for use in humans. It is intended for research purposes only. To the best of our knowledge, the chemical, physical, and toxicological properties of this material have not been established.

Emergency Overview: Irritant. Irritating to eyes, respiratory system, skin.

Potential Health Effects:

Inhalation: May be harmful if inhaled. May cause respiratory tract irritation.

Eye Contact: May cause eye irritation.

Skin Contact: May be harmful if absorbed through skin. Prolonged or repeated contact may cause skin irritation.

Ingestion: May be harmful if swallowed.

IV. First Aid Measures:

Inhalation: Remove to fresh air. If breathing is difficult, get medical attention.

Ingestion: If person is conscious, wash out mouth with water. Get medical attention.

Skin exposure: Wash skin with soap and water. If irritation develops or persists, get medical attention.

Eye exposure: Immediately flush eyes water for at least 15 minutes. Get medical attention.

V. Fire Fighting Measures:

Flash Point: N/A

Autoignition Temperature: N/A

Explosion: N/A

Fire extinguishing media: water spray, dry chemical, alcohol foam, or carbon dioxide.

Firefighting: wear protective clothing and self-contained breathing apparatus to prevent contact with skin and eyes. May emit toxic fumes under fire conditions.

VI. Accidental Release Measures:

Wear appropriate personal protective equipment as indicated in Section VIII. Absorb liquid with an absorbent material. Transfer contaminated absorbent to a closed chemical waste container for disposal. Wash spill site after material has been picked up for disposal.

VII. Handling And Storage:

Store at 4°C in tightly closed container.

Avoid inhalation of vapor or mist. Avoid contact with eyes, skin, and clothing. Wash thoroughly after handling. Avoid prolonged or repeated exposure.

VIII. Exposure Controls/Personal:

Ventilation System: a system of local and/or general exhaust is recommended.

Skin Protection: wear compatible chemical resistant gloves and protective clothing.

Eye protection: wear protective safety glasses or chemical safety goggles. Maintain eye wash fountain and quick-drench facilities in work area.

IX. Physical And Chemical Properties:

Appearance:	clear faint yellow colored liquid
Odor:	data not available
pH:	data not available
Boiling Point:	>100°C/212°F (water)
Melting or Freezing Point:	<0°C/32°F (water)
Flash Point:	data not available
Volatile Organic Compounds (VOC):	data not available
Autoignition temp.	data not available
Solubility (water)	miscible in water

X. Stability and Reactivity:

Stability: Stable under normal conditions.

Conditions to avoid: strong oxidizing agents, strong acids, strong bases.

Hazardous Decomposition: carbon monoxide, carbon dioxide.

Hazardous polymerization: will not occur.

XI. Toxicological Information:

Acute toxicity: data not available. Chronic exposure: data not available

Potential Health Effects:

Inhalation: May be harmful if inhaled. Causes respiratory tract irritation.

Skin: May be harmful if absorbed through skin. Causes skin irritation.

Eyes: Causes eye irritation.

Ingestion: Harmful if swallowed.

Toxicity Data on Hazardous ingredient Dimethyl Sulfoxide, CAS#67-68-5

RTECS: PV6210000

LD50 Oral rat 14,500 mg/kg

LC50 Inhalation rat 4 h 40250 ppm

LD50 Dermal rabbit > 5,000 mg/kg

XII. Ecological Information:

No data available.

XIII. Disposal Considerations: Dispose of in accordance with federal, state and local environmental regulations. Contact a licensed professional waste disposal service to dispose of this material.

XIV. Transport Information:

D.O.T. Proper Shipping Name: None. This substance is considered non-hazardous for transport.

IATA Proper Shipping Name: None. This substance is considered non-hazardous for air transport.

XV. Regulatory Information:

EU: Not classified

OSHA: Ingredient Dimethyl Sulfoxide, CAS#67-68-5: Combustible Liquid, Target Organ Effect

Canadian DSL: Listed: Ingredient Dimethyl Sulfoxide, CAS#67-68-5

SARA 302, 313 Ingredients Not Listed.

SARA 311/312: Ingredient Dimethyl Sulfoxide, CAS#67-68-5: Fire Hazard, Chronic Health Hazard.

Massachusetts Right To Know: Ingredients Not Listed.

Pennsylvania Right To Know: Ingredient Dimethyl Sulfoxide, CAS#67-68-5

New Jersey Right To Know: Ingredient Dimethyl Sulfoxide, CAS#67-68-5

California Prop. 65: Ingredients Not Listed.

XVI. Other Information:

This product is for research use only and is not intended for use in humans. To the best of our knowledge, this document is accurate. It is intended to serve as a guide for safe use of this product in a laboratory setting by experienced personnel. The burden of safe use of this material rests entirely with the user. The above information is believed to be accurate but is not necessarily all-inclusive and shall be used only as a guide. Cell Signaling Technology, Inc., shall not be held liable for any damage resulting from the handling of or from contact with the above product.

Material Safety Data Sheet (MSDS) for Antibodies

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.
Hazardous 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.