

Store at
-20°C
#5302

PathScan® Multiplex Western Cocktail II: Phospho-p90RSK, Phospho-p53, Phospho-p38 MAPK and Phospho-S6 Ribosomal Protein Detection Cocktail II

www.cellsignal.com

Support: 877-678-TECH (8324)
www.cellsignal.com/support

Orders: 877-616-CELL (2355)
orders@cellsignal.com

250 µl (5 western blots)

rev. 01/29/16

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

Applications W	Species Cross-Reactivity H, M, R, Mi	Source Rabbit
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Antibody Cocktail Components and Molecular Weights		
No.	Antibody	Molecular Weight
4858	Phospho-S6 Ribosomal Protein (Ser235/236) (D57.2.2E) XP® Rabbit mAb	32 kDa
9284	Phospho-p53 (Ser15) Antibody	53 kDa
4511	Phospho-p38 MAPK (Thr180/Tyr182) (D3F9) XP® Rabbit mAb	43 kDa
11989	Phospho-p90RSK (Ser380) (D3H11) Rabbit mAb	90 kDa
9742	eIF4E Antibody	25 kDa

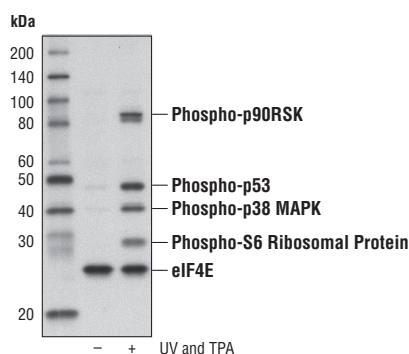
Description: The PathScan® Multiplex Western Cocktail II offers a unique method to assay the activation of multiple pathways on one membrane without stripping and reprobing. This method saves the user valuable time, while increasing accuracy and minimizing reagent waste. The system allows the user to simultaneously detect levels of phospho-p90RSK, phospho-p53, phospho-p38 MAPK and phospho-S6 ribosomal protein. The kit also includes eIF4E antibody to control protein loading.

Specificity/Sensitivity: Each phospho-antibody in this cocktail recognizes endogenous levels of only the phosphorylated form of its specific target. The eIF4E antibody detects endogenous levels of its target protein independent of phosphorylation and is provided to control for protein loading.

Source/Purification: Polyclonal antibodies are produced by immunizing animals with synthetic peptides. Antibodies are purified by protein A and peptide affinity chromatography. Monoclonal antibodies are produced by immunizing animals with a synthetic peptide.

Background: The 90 kDa ribosomal S6 kinases (RSK1-3) are a family of serine/threonine kinases broadly expressed in response to many growth factors, polypeptide hormones and neurotransmitters (1). p90RSK is activated by Erk1 and Erk2 in vitro and in vivo via phosphorylation (2). Several sites, such as Ser380, Thr359 and Ser363, are important for its activation (3).

The p53 tumor suppressor protein plays a major role in cellular response to DNA damage and other genomic aberrations. Activation of p53 can lead to either cell cycle arrest and DNA repair or apoptosis (4). p53 is phosphorylated at multiple sites in vivo and by several different protein kinases in vitro (5,6). DNA damage induces phosphorylation of p53 at Ser15 and Ser20 and leads to reduced interaction of p53 with its negative regulator, oncoprotein MDM2 (7).



Western blot analysis of extracts from Mv1Lu mink lung epithelial cells untreated or treated with UV and TPA, using PathScan Multiplex Western cocktail II to detect phosphorylation of p90RSK, p53, p38 MAPK and S6 ribosomal protein.

p38 MAP kinase controls cellular responses to cytokines and stress (8-11). Like the SAPK/JNK pathway, p38 MAP kinase is activated by a variety of cellular stresses including osmotic shock, inflammatory cytokines, lipopolysaccharides (LPS), UV light and growth factors (8-12). MKK3, MKK6 and SEK activate p38 MAP kinase by phosphorylation at Thr180 and Tyr182.

Growth factors and mitogens induce the activation of p70 S6 kinase, which in turn phosphorylates the S6 ribosomal protein. Phosphorylation of S6 correlates with an increase in translation, particularly of mRNAs with an oligopyrimidine tract in their 5' untranslated regions (13). This group of mRNAs (5'TOP) encodes proteins involved in cell cycle progression and proteins that are part of the translational machinery, such as ribosomal proteins and elongation factors (13,14).

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.

Recommended Antibody Dilutions:
Western blotting 1:200

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) Frodin, M. and Gammeltoft, S. (1999) *Mol. Cell. Endocrinol.* 151, 65-77.
- (2) Lazar, D.F. et al. (1995) *J. Biol. Chem.* 270, 20801-20807.
- (3) Dalby, K.N. et al. (1998) *J. Biol. Chem.* 273, 1496-1505.
- (4) Levine, A.J. (1997) *Cell* 88, 323-331.
- (5) Meek, D.W. (1994) *Semin. Cancer Biol.* 5, 203-210.
- (6) Milczarek, G.J. et al. (1997) *Life Sci.* 60, 1-11.
- (7) Shieh, S.Y. et al. (1997) *Cell* 91, 325-334.
- (8) Han, J. et al. (1994) *Science* 265, 808-811.
- (9) Lee, J.C. et al. (1994) *Nature* 372, 739-746.
- (10) Rouse, J. et al. (1994) *Cell* 78, 1027-1037.
- (11) Freshney, N.W. et al. (1994) *Cell* 78, 1039-1049.
- (12) Raingeaud, J. et al. (1995) *J. Biol. Chem.* 270, 7420-7426.
- (13) Peterson, R.T. and Schreiber, S.L. (1998) *Curr. Biol.* 8, R248-R250.
- (14) Jefferies, H.B. et al. (1997) *EMBO J.* 16, 3693-3704.

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.

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.

- 1. 20X Phosphate Buffered Saline (PBS):** (#9808) To prepare 1 L 1X PBS: add 50 ml 20X PBS to 950 ml dH₂O, mix.
- 2. 10X Tris Buffered Saline (TBS):** (#12498) To prepare 1 L 1X TBS: add 100 ml 10X to 900 ml dH₂O, mix.
- 3. 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.
- 4. 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.
- 5. 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.
- 6. 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.
- 7. Nonfat Dry Milk:** (#9999)
- 8. 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.
- 9. Wash Buffer:** (#9997) 1X TBST
- 10. Bovine Serum Albumin (BSA):** (#9998)
- 11. 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.
- 12. Biotinylated Protein Ladder Detection Pack:** (#7727)
- 13. Prestained Protein Marker, Broad Range (Premixed Format):** (#7720)
- 14. Blotting Membrane and Paper:** (#12369) This protocol has been optimized for nitrocellulose membranes. Pore size 0.2 µm is generally recommended.
- 15. Secondary Antibody Conjugated to HRP:** anti-rabbit (#7074); anti-mouse (#7076)
- 16. Detection Reagent:** LumiGLO® chemiluminescent reagent and peroxide (#7003) or SignalFire™ ECL Reagent (#6883)

B. Protein Blotting

A general protocol for sample preparation.

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 for a 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 sec to complete cell lysis and shear DNA (to reduce sample viscosity).
5. Heat a 20 µl sample to 95–100°C for 5 min; cool on ice.
6. Microcentrifuge for 5 min.
7. 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.
8. 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

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

II. Primary Antibody Incubation

1. 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.
2. Wash three times for 5 min each with 15 ml of TBST.
3. 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.
4. Wash three times for 5 min each with 15 ml of TBST.
5. Proceed with detection (Section D).

D. Detection of Proteins

1. 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.
2. 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.

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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.