#5301 Store at -20°C

PathScan® Multiplex Western Cocktail I:

Phospho-p90RSK, Phospho-Akt, Phospho-p44/42 MAPK and Phospho-S6 Ribosomal Protein Detection Cocktail I

☑ 250 µl
(5 western blots)

rev. 01/29/16

Cell Signaling TECHNOLOGY®

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

Applications	Species Cross-Reactivity*	Source
W Endogenous	H, M, R	Rabbit**

Antibody Cocktail Components and Molecular Weights

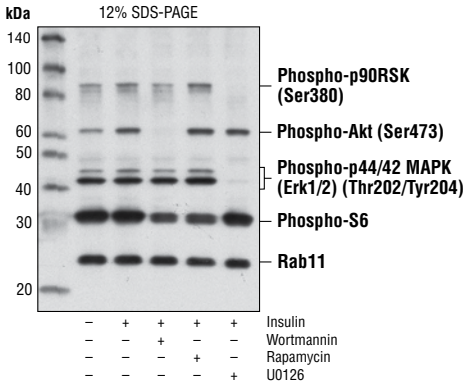
No.	Antibody	Molecular Weight
9335	Phospho-p90RSK (Ser380) (9D9) Rabbit mAb	90 kDa
4060	Phospho-Akt (Ser473) (D9E) XP® Rabbit mAb	60 kDa
4370	Phospho-p44/42 MAPK (Erk1/2) (Thr202/Tyr204) (D13.14.4E) XP® Rabbit mAb	42, 44 kDa
4858	Phospho-S6 Ribosomal Protein (Ser235/236) (D57.2.2E) Rabbit mAb	32 kDa
5589	Rab11 (D4F5) XP® Rabbit mAb	25 kDa

Description: The PathScan® Multiplex Western Cocktail I 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-Akt, phospho-p44/42 MAPK (Erk1/2) and phospho-S6 ribosomal protein. The cocktail also includes the Rab11 Antibody to control for protein loading.

Background: Akt, also referred to as PKB or Rac, plays a critical role in controlling the balance between survival and apoptosis (1-3). This protein kinase is activated by insulin and various growth and survival factors, and functions in a wortmannin-sensitive pathway involving PI3 kinase (2,3). Akt is activated by phospholipid binding and activation loop phosphorylation at Thr308 by PDK1 (4) and also by phosphorylation within the carboxy-terminus at Ser473.

Both p44 and p42 MAP kinases (Erk1 and Erk2) play a critical role in the regulation of cell growth and differentiation (5-8). MAP kinases are activated by a wide variety of extracellular signals including growth and neurotrophic factors, cytokines, hormones and neurotransmitters. Activation of MAP kinases occurs through phosphorylation of threonine and tyrosine (202 and 204 of human MAP kinase [Erk1] or 183 and 185 of rat Erk2) at the sequence T*EY* by a single upstream MAP kinase kinase (MEK) (9,10). One of the downstream targets of p44/42 MAPK is p90RSK.

To effectively promote growth and cell division in a sustained manner, growth factors and mitogens must upregulate translation (11,12). 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 (12).



Western blot analysis of extracts from CHO cells, untreated or insulin-treated following pretreatment with wortmannin (PI3 kinase inhibitor), rapamycin (mTOR inhibitor) and/or U0126 (MEK inhibitor) as indicated, using PathScan® Multiplex Western Cocktail I to detect phosphorylation of p90RSK, Akt, p44/42 MAPK and S6 ribosomal protein.

Specificity/Sensitivity: Each phospho-antibody in this cocktail recognizes endogenous levels of only the phosphorylated form of its specific target. The Rab11 antibody detects endogenous levels of its target protein independent of phosphorylation, and is provided to control for protein loading.

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

Storage: 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 antibody.*

***Species cross-reactivity is determined by western blot, using the individual antibody cocktail components.**

****Anti-rabbit secondary antibodies must be used to detect this antibody.**

Recommended Antibody Dilutions:
Western blotting 1:200

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

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

Background References:

(1) Franke, T.F. (1997) *Cell* 88, 435–437.
(2) Burgering, B.T. and Coffer, P.J. (1995) *Nature* 376, 599–602.
(3) Franke, T.F. et al. (1995) *Cell* 81, 727–736.
(4) Alessi, D.R. et al. (1996) *EMBO J.* 15, 6541–6551.
(5) Marshall, C.J. (1995) *Cell* 80, 179–185.
(6) Hunter, T. (1995) *Cell* 80, 225–236.
(7) Hill, C.S. and Treisman, R. (1995) *Cell* 80, 199–211.
(8) Cowley, S. et al. (1994) *Cell* 77, 841–852.
(9) Sturgill, T.W. et al. (1988) *Nature* 334, 715–718.
(10) Payne, D.M. et al. (1991) *EMBO J.* 10, 885–892.
(11) Dufner, A. and Thomas, G. (1999) *Exp. Cell. Res.* 253, 100–109.
(12) Peterson, R.T. and Schreiber, S.L. (1998) *Curr. Biol.* 8, R248–R250.

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 **All**—all species expected Species enclosed in parentheses are predicted to react based on 100% homology.

#5301

Western Immunoblotting Protocol (Primary Ab Incubation In BSA)

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.

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. Bovine Serum Albumin (BSA)
9. **Primary Antibody Dilution Buffer:** 1X TBS, 0.1% Tween-20 with 5% BSA; for 20 ml, add 2 ml 10X TBS to 18 ml water, mix. Add 1.0 g BSA and mix well. While stirring, add 20 µl Tween-20 (100%).
10. **Phototope®-HRP Western Blot Detection System** #7071**:** Includes biotinylated protein ladder, secondary anti-rabbit (#7074) antibody conjugated to horseradish peroxidase (HRP), anti-biotin antibody conjugated to HRP, LumiGLO® chemiluminescent reagent and peroxide.
11. Prestained Protein Marker, Broad Range (Premixed Format) #7720
12. Biotinylated Protein Ladder Detection Pack #7727
13. **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 markers (#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.

SAFETY DATA SHEET (SDS): According to the OSHA Hazard Communication Standard 29 CFR 1910.1200
Issuing Date: 2014-06-20 **Revision Date:** 2014-07-29 **Version:** 1

SECTION 1. Identification

Product identifier

Product number 5301
Product name Phospho-Jak3 (Tyr980/981) (D44E3) Rabbit mAb

Recommended use of the chemical and restrictions on use

Identified uses This product is intended for research purposes only.
Uses advised against This product is not intended for use in diagnostic procedures or therapeutics. This product is not intended for use in humans or animals.

Manufacturer, importer, supplier

Manufacturer address Cell Signaling Technology, Inc.
3 Trask Lane
Danvers, MA 01923
United States
TEL: +1 978 867 2300
FAX: +1 978 867 2400
Website www.cellsignal.com
Email address support@cellsignal.com
Company phone number 978-867-2300
Emergency telephone number In case of emergency call CHEMTREC 1-800-424-9300

SECTION 2. Hazard(s) identification

Classification

This substance/mixture is not considered hazardous by the 2012 OSHA Hazard Communication Standard (29 CFR 1910.1200)

GHS Label elements, including precautionary statements

Signal Word
None

Hazard statement(s)
None

Precautionary Statement(s)
None

Supplementary Hazard Information

Hazards not otherwise classified (HNOC) None.

SECTION 3. Composition/information on ingredients

Chemical nature Aqueous buffer solution

Chemical Name	CAS No	Weight %
glycerol	56-81-5	30-60
sodium chloride	7647-14-5	0.1-1
sodium 4-(2-hydroxyethyl)piperazin-1-ylethanesulphonate	75277-39-3	0.1-1

SECTION 4. First-aid measures

Eye contact Rinse thoroughly with plenty of water, also under the eyelids. Keep eye wide open while rinsing.
Skin contact Consult a physician if necessary. Wash off immediately with soap and plenty of water removing all contaminated clothes and shoes.
Inhalation Move to fresh air. Consult a physician. If not breathing, give artificial respiration.
Ingestion Rinse mouth. Drink plenty of water. If symptoms persist, call a physician. Do NOT induce vomiting. If swallowed, do not induce vomiting - seek medical advice. Never give anything by mouth to an unconscious person.

Most important symptoms and effects, both acute and delayed

No information available.

Indication of any immediate medical attention and special treatment needed

Treat symptomatically.

Advice for emergency responders

General advice If symptoms persist, call a physician. Show this safety data sheet to the doctor in attendance. Do not breathe dust/fume/gas/mist/vapors/spray.
Protection of first-aiders Use personal protective equipment.

SECTION 5. Fire-fighting measures

Extinguishing media

Suitable Extinguishing Media Use extinguishing measures that are appropriate to local circumstances and the surrounding environment.
Unsuitable Extinguishing Media CAUTION: Use of water spray when fighting fire may be inefficient.

Specific hazards arising from the chemical

No information available.

Explosion Data

Sensitivity to Mechanical Impact None.
Sensitivity to Static Discharge None.

Protective Equipment and Precautions for Firefighters

As in any fire, wear self-contained breathing apparatus pressure-demand, MSHA/NIOSH (approved or equivalent) and full protective gear.

SECTION 6. Accidental release measures

Personal precautions, protective equipment and emergency procedures

For non-emergency personnel Ensure adequate ventilation.
Other information No information available.

Environmental precautions

Prevent entry into waterways, sewers, basements or confined areas. Do not flush into surface water or sanitary sewer system.

Methods and material for containment and cleaning up

Methods for containment Prevent further leakage or spillage if safe to do so.
Methods for cleaning up Cover liquid spill with sand, earth or other noncombustible absorbent material. Cover powder spill with plastic sheet or tarp to minimize spreading. Pick up and transfer to properly labeled containers.

SECTION 7. Handling and storage

Precautions for safe handling

Avoid contact with skin, eyes and clothing. Wear personal protective equipment. Prevent the formation of vapors, mists and aerosols. Do not eat, drink or smoke when using this product.

Conditions for safe storage, including any incompatibilities

Technical measures/Storage conditions Keep container tightly closed in a dry and well-ventilated place. Keep out of the reach of children.
Packaging material No information available.
Incompatible products Strong oxidizing agents.

SECTION 8. Exposure controls/personal protection

Control parameters

Occupational exposure limit values			
Chemical Name	**ACGIH TLV**	**OSHA PEL**	**NIOSH REL**
glycerol	-	TWA mist, total particulate: 15 mg/m³ TWA mist, respirable fraction: 5 mg/m³	-

Appropriate engineering controls

Showers, eyewash stations, and ventilation systems.

Individual protection measures, such as personal protective equipment

Personal protective equipment (PPE) needs to be selected depending on the implemented engineering controls, frequency/duration of work activities and the concentrations of the hazardous substance.

Eye/face protection Tightly fitting safety goggles.
Skin and body protection Wear protective gloves/clothing.
Respiratory protection If exposure limits are exceeded or irritation is experienced, NIOSH/MSHA approved respiratory protection should be worn. Positive-pressure supplied air respirators may be required for high airborne contaminant concentrations. Respiratory protection must be provided in accordance with current local regulations.
Hygiene measures Handle in accordance with good industrial hygiene and safety practice.

SECTION 9. Physical and chemical properties

Information on basic physical and chemical properties

Physical state Liquid
Appearance Clear
Odor No information available
Color Colorless
Odor Threshold No information available
pH 7.4 @ 20 °C
Melting point/freezing point No information available
Initial boiling point and boiling range No information available
Flash point No information available.
Evaporation rate No information available
Flammability (solid, gas) No information available
Upper flammability limit No information available.
Lower flammability limit No information available.
Vapor pressure No information available
Vapor density No information available
Relative density No information available
Solubility No information available.
Solubility in other solvents No information available
Partition coefficient: n-octanol/water No information available
Autoignition temperature No information available
Decomposition temperature No information available.
Explosive properties No information available
Oxidizing properties No information available
VOC content No information available
Viscosity No information available.
Density No information available.

SECTION 10. Stability and reactivity

Reactivity

No information available.

Chemical stability

Stable under recommended storage conditions.

Possibility of hazardous reactions

Hazardous reactions None under normal processing.
Hazardous polymerization None under normal processing.

Conditions to Avoid

No information available.

Incompatible Materials

Strong oxidizing agents.

Hazardous Decomposition Products

Thermal decomposition can lead to release of irritating gases and vapors: Acrolein. Hydrogen chloride.

SECTION 11. Toxicological information

Information on likely routes of exposure

Inhalation Mist may be injurious to the kidneys at very high exposure levels. Not an expected route of exposure.
Eye contact Contact with eyes may cause mild irritation.

Skin contact No known hazard in contact with skin.
Ingestion Ingestion may cause gastrointestinal irritation, nausea, vomiting and diarrhea.

Information on toxicological effects

This material should only be handled by, or under the close supervision of, those properly qualified in the handling and use of potentially hazardous chemicals. It should be borne in mind that the toxocological and physiological properties of this compound is not well defined.

Chemical Name	LD50 Oral	LD50 Dermal	LC50 Inhalation
glycerol	12600 mg/kg (Rat)	> 10000 mg/kg (Rabbit)	-
sodium chloride	3000 mg/kg (Rat)	> 10000 mg/kg (Rabbit)	> 42000 mg/m³ (Rat) 1 h

Delayed and immediate effects as well as chronic effects from short and long-term exposure

Symptoms No information available.
Serious eye damage/eye irritation Mild / Nonirritant: OECD TG 437 (Bovine Corneal Opacity and Permeability (BCOP)).
Sensitization No information available.
Mutagenic effects No information available.
Carcinogenicity No component of this product present at levels greater than or equal to 0.1% is identifiable as probable, possible or confirmed carcinogen by IARC, ACGIH, NTP, or OSHA.

Reproductive toxicity No information available.
STOT - single exposure No information available.
STOT - repeated exposure No information available.
Target Organ Effects Eyes, Respiratory system, Kidney.
Neurological effects No information available.
Aspiration Hazard No information available.
Other information glycerol: NOAEL (inhalation: 6 h/d, 5d/week) = 0.167 mg/L (Rat) 13 weeks
glycerol: LOAEL (inhalation: 6 h/d, 5d/week) = 1-4 mg/L (Rat) 2 weeks
(Source: IUCLID / Method: Directive 87/302/EEC, part B "Sub-chronic inhalation toxicity study: 90-day repeated dose study using rodent species")

SECTION 12. Ecological information

Ecotoxicity

Product does not present an aquatic toxicity hazard based on known or supplied information.

Chemical Name	Toxicity to algae	Toxicity to fish	Toxicity to daphnia and other aquatic invertebrates
glycerol	-	LC50 51 - 57 mL/L (Oncorhynchus mykiss) 96 h	EC50 500 mg/L (Daphnia magna) 24 h
sodium chloride	-	LC50 4747 - 7824 mg/L (Oncorhynchus mykiss) 96 h LC50 12946 mg/L (Lepomis macrochirus) 96 h LC50 5560 - 6080 mg/L (Lepomis macrochirus) 96 h LC50 6420 - 6700 mg/L (Pimephales promelas) 96 h LC50 7050 mg/L (Pimephales promelas) 96 h LC50 6020 - 7070 mg/L (Pimephales promelas) 96 h	EC50 340.7 - 469.2 mg/L (Daphnia magna) 48 h EC50 1000 mg/L (Daphnia magna) 48 h

Persistence and degradability Product is biodegradable.
Bioaccumulation Not likely to bioaccumulate.
Mobility Will likely be mobile in the environment due to its water solubility

Chemical Name	Octanol-Water Partition Coefficient
glycerol	-1.76

Other adverse effects

No information available.

SECTION 13. Disposal considerations

Waste Disposal Methods

Dispose of in accordance with all applicable national environmental laws and regulations.

Disposal considerations

Do not empty into drains; dispose of this material and its container in a safe way.

SECTION 14. Transport information

This material is not subject to regulation as a hazardous material for shipping.

SECTION 15. Regulatory information

North American Inventory Listing

Chemical Name	TSCA 8(b)	TSCA 12(b)	DSL	NDSL
glycerol	Listed	Not Listed	Listed	Not Listed
sodium chloride	Listed	Not Listed	Listed	Not Listed
sodium 4-(2-hydroxyethyl)piperazin-1-yl ethanesulphonate	Listed	Not Listed	Not Listed	Listed

Canadian Workplace Hazardous Materials Information System (WHMIS) Classification

This product does not meet the criteria for classification under the Hazardous Products Act.

SARA 313

Section 313 of Title III of the Superfund Amendments and Reauthorization Act of 1986 (SARA). This product does not contain any chemicals which are subject to the reporting requirements of the Act and Title 40 of the Code of Federal Regulations, Part 372.

SARA 311/312 Hazard Categories

Acute Health Hazard No
Chronic Health Hazard No
Fire Hazard No
Sudden Release of Pressure Hazard No
Reactive Hazard No

Clean Water Act

This product does not contain any substances regulated as pollutants pursuant to the Clean Water Act (40 CFR 122.21 and 40 CFR 122.42).

CERCLA

This material, as supplied, does not contain any substances regulated as hazardous substances under the Comprehensive Environmental Response Compensation and Liability Act (CERCLA) (40 CFR 302) or the Superfund Amendments and Reauthorization Act (SARA) (40 CFR 355). There may be specific reporting requirements at the local, regional, or state level pertaining to releases of this material.

California Proposition 65

This product does not contain any Proposition 65 chemicals.

U.S. State Right-to-Know Regulations

This product contains the following U.S. State Right to Know chemicals:

Chemical Name	New Jersey	Massachusetts	Pennsylvania
glycerol	Listed	Listed	Listed

U.S. FIFRA Label Information

This product does not contain any substances regulated as pesticides.

US Commerce Department - Export Administration Regulations Information

This product does not contain any substances regulated under the Chemical Weapons Convention (CWC).

U.S. Drug Enforcement Administration Information

This product does not contain any substances regulated under the DEA.

SECTION 16. Other information

Issuing Date: 2014-06-20
Revision Date: 2014-07-29
Disclaimer

The information provided in this Safety Data Sheet is correct to the best of our knowledge, information and belief at the date of its publication. The information given is designed only as a guidance for safe handling, use, processing, storage, transportation, disposal and release and is not to be considered a warranty or quality specification. The information relates only to the specific material designated and may not be valid for such material used in combination with any other materials or in any process, unless specified in the text.

End of Safety Data Sheet