

PhosphoPlus® Akt (Ser473) In-Cell Duet (ICW Compatible)



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

Support ■ 877-678-TECH (8324)
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- Small Kit
(1 x 96 well plate)
- Large Kit
(5 x 96 well plates)

rev. 02/22/16

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

Products Included	Product #	Volume	Applicaton	Dilution	Species Cross-Reactivity
Primary Cocktail	5532	500 µl	ICW	1:10	H, M, R, Mk
Detection Cocktail	5531	500 µl	ICW	1:10	N/A

Kit Analytes	Detection Dye	Ex _(max) (nm)	Em _(max) (nm)
Phospho-Akt (Ser473)	DyLight® 800	777	794
Total Akt	DyLight® 680	692	712

Description: PhosphoPlus® Akt (Ser473) In-Cell Duet from Cell Signaling Technology (CST) provides an easy method to assess protein activation status using a multi-well plate scanner with near infrared detection capabilities, such as the LI-COR® Biosciences Odyssey® Infrared Imaging System. This kit contains a pre-optimized activation-state and total protein antibody cocktail, selected based on superior performance. Phosphorylated and total protein are detected simultaneously in the same well, allowing levels of phosphorylated protein to be normalized to total protein. A near infrared detection cocktail is also included.

Specificity/Sensitivity: Phospho-Akt (Ser473) antibody detects endogenous levels of Akt only when phosphorylated at Ser473. Total Akt detects endogenous levels of total Akt protein. This antibody does not cross-react with other related proteins.

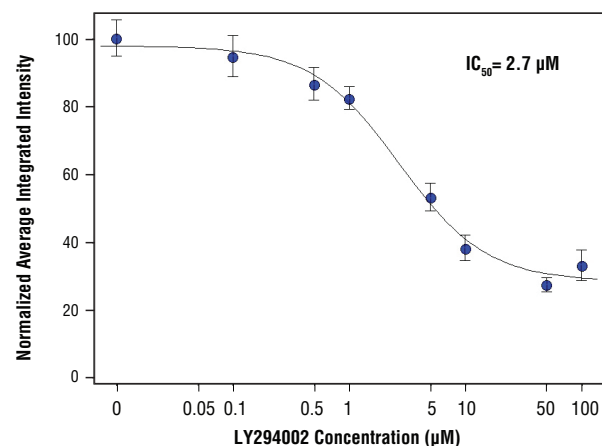
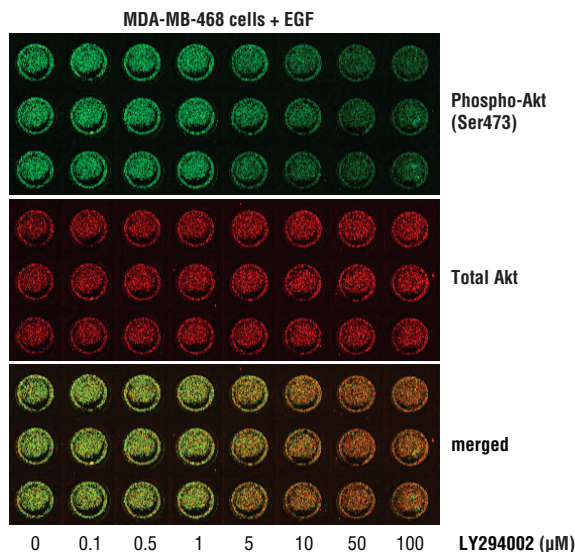
Source/Purification: Monoclonal antibodies are produced by immunizing animals with synthetic phosphopeptides corresponding to residues surrounding Ser473 of human Akt or at the carboxy terminal sequence of human Akt.

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 either cocktail.*

Species cross-reactivity is determined by western blot with parent antibodies.

DyLight® is a trademark of Thermo Fisher Scientific Inc. and its subsidiaries.

LI-COR® and Odyssey® are registered trademarks of LI-COR Biosciences.



Analysis of MDA-MB-468 cells exposed to varying concentrations of LY294002 (PI3 Kinase Inhibitor) #9901 for 2 hours, followed by hEGF #8916 stimulation for 20 minutes. The phosphorylation status of Akt, as well as the total protein expression level, was measured simultaneously using the PhosphoPlus® Akt (Ser473) In-Cell Duet (ICW Compatible) #7255. With increasing concentrations of LY294002, a significant decrease (~3-fold) in phospho-Akt signal as compared to the hEGF-stimulated control was observed, while total Akt protein levels remained unchanged and were used to normalize the data. When using phospho-Akt as a measurement, the IC₅₀ of this compound was 2.7 µM. Data and images were generated using the LI-COR® Biosciences Odyssey® Infrared Imaging System

Applications Key: ICW—In-Cell Western

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.

Background: Akt, also referred to as PKB or Rac, plays a critical role in controlling survival and apoptosis (1-3). This protein kinase is activated by insulin and various growth and survival factors to function 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 by phosphorylation within the carboxy terminus at Ser473. The previously elusive PDK2 responsible for phosphorylation of Akt at Ser473 has been identified as mammalian target of rapamycin (mTOR) in a rapamycin-insensitive complex with rictor and Sin1 (5,6). Akt promotes cell survival by inhibiting apoptosis through phosphorylation and inactivation of several targets, including Bad (7), forkhead transcription factors (8), c-Raf, (9) and caspase-9. PTEN phosphatase is a major negative regulator of the PI3 kinase/Akt signaling pathway (10). LY294002 is a specific PI3 kinase inhibitor (11). Another essential Akt function is the regulation of glycogen synthesis through phosphorylation and inactivation of GSK-3 α and β (12,13). Akt may also play a role in insulin stimulation of glucose transport (12). In addition to its role in survival and glycogen synthesis, Akt is involved in cell cycle regulation by preventing GSK-3 β mediated phosphorylation and degradation of cyclin D1 (14) and by negatively regulating the cyclin dependent kinase inhibitors p27 Kip (15) and p21 Waf1/CIP1 (16). Akt also plays a critical role in cell growth by directly phosphorylating mTOR in a rapamycin-sensitive complex containing raptor (17). More importantly, Akt phosphorylates and inactivates tuberlin (TSC2), an inhibitor of mTOR within the mTOR-raptor complex (18). Inhibition of mTOR stops the protein synthesis machinery by inactivating p70 S6 kinase and activating the eukaryotic initiation factor 4E binding protein 1 (4E-BP1), an inhibitor of translation (18,19).

Background References:

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- (6) Jacinto, E. et al. (2006) *Cell* 127, 125-37.
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- (12) Hajdуч, E. et al. (2001) *FEBS Lett* 492, 199-203.
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- (16) Zhou, B.P. et al. (2001) *Nat Cell Biol* 3, 245-52.
- (17) Navé, B.T. et al. (1999) *Biochem J* 344 Pt 2, 427-31.
- (18) Inoki, K. et al. (2002) *Nat Cell Biol* 4, 648-57.
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PhosphoPlus® In-Cell Duet (ICW Compatible) Protocol

A Solutions and Reagents

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

- 1. 10X Phosphate Buffered Saline (PBS):** To prepare 1 L add 80 g sodium chloride (NaCl), 2 g potassium chloride (KCl), 14.4 g sodium phosphate, dibasic (Na_2HPO_4) and 2.4 g potassium phosphate, monobasic (KH_2PO_4) to 1 L dH_2O . Adjust pH to 7.4.
- 2. Formaldehyde,** use fresh, dilute in PBS for use.
- 3. Blocking Buffer (1X PBS/5% normal goat serum/0.3% Triton X-100):** To prepare 25 ml, add 2.5 ml 10X PBS, 1.25 ml normal goat serum and 21.25 ml dH_2O and mix well. While stirring, add 75 μl Triton X-100.
- 4. Antibody Dilution Buffer (1X PBS/1% BSA/0.3% Triton X-100):** To prepare 25 ml, add 2.5 ml 10X PBS to 22.5 ml dH_2O , mix. Add 0.25 g BSA and mix well. While stirring, add 75 μl Triton X-100.

B Specimen Preparation

NOTE: Cells should be grown, treated, fixed, and stained directly in multi-well plates.

1. Aspirate culture medium, and then cover cells to a depth of 2–3 mm with 4% formaldehyde diluted in 1X PBS.
NOTE: Formaldehyde is toxic, use only in fume hood.
2. Allow cells to fix for 15 minutes at room temperature.
3. Aspirate fixative, rinse three times in PBS for 5 minutes each.
4. Proceed with immunostaining.

C Immunostaining

NOTE: Include control well(s) for detection cocktail staining alone (no primary cocktail) for nonspecific background correction.

1. Block specimen in Blocking Buffer for 60 minutes.
2. While blocking, prepare primary cocktail by diluting as indicated on datasheet in Antibody Dilution Buffer.
3. Aspirate blocking solution, apply diluted primary cocktail.
4. Incubate overnight at 4°C.
5. Rinse three times in PBS for 5 minutes each.
6. Prepare detection cocktail by diluting as indicated on datasheet in Antibody Dilution Buffer.
7. Incubate 1-2 hours at room temperature in the dark.
8. Rinse three times in PBS for 5 minutes each.
9. For best results examine specimens immediately using appropriate excitation wavelengths.

SDS #: 7255
Revision Date: 2010-08-24
Version 5.01

1. PRODUCT AND COMPANY IDENTIFICATION

Product Code(s) 7255, 7257, 7261, 7263
Product Name PhosphoPlus® In-Cell Duets (ICW Compatible)

Pure substance/preparation Substance Preparation

Identified uses For research use only, Not for use in humans.

Manufacturer
Cell Signaling Technology, Inc.
3 Trask Lane
Danvers, MA 01923
TEL: 978-867-2300

2. HAZARDS IDENTIFICATION

Emergency Overview
The product contains no substances which at their given concentration, are considered to be hazardous to health.
Physical State liquid
Odor No information available

Potential Health Effects

Acute Toxicity
Eyes May cause slight irritation.
Skin No known effect based on information supplied.
Inhalation No known effect based on information supplied.
Ingestion No known effect based on information supplied.

Chronic Effects

Chronic toxicity No known effect based on information supplied.
Aggravated Medical Conditions None known.
Environmental hazard See Section 12 for additional Ecological Information.

3. COMPOSITION/INFORMATION ON INGREDIENTS

Hazardous	Chemical Name	CAS-No	Weight %
	Glycerol	56-81-5	30 - 60

Chemical Name	CAS-No	Weight %	EC-No
Sodium HEPES	75277-39-3	0.1 - 1	-
Sodium chloride	7647-14-5	0.1 - 1	231-598-3
Bovine Serum Albumin	9049-46-8	< 0.1	-
Glycerol	56-81-5	30 - 60	200-289-5

4. FIRST AID MEASURES

Eye contact Rinse immediately with plenty of water and seek medical advice.

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Respiratory protection None required under normal usage. If exposure limits are exceeded or irritation is experienced, NIOSH/MSHA approved respiratory protection should be worn. Respiratory protection must be provided in accordance with current local regulations.

Eye/Face Protection Safety glasses with side-shields

Skin and body protection Protective gloves.

9. PHYSICAL AND CHEMICAL PROPERTIES

Physical State liquid
Appearance aqueous solution
Color clear
Odor No information available
pH 7.4
Solubility No information available
Melting point/range No information available
Boiling Point/Range No information available
Flash point No information available
Autoignition temperature No information available
Flammability Limits in Air No information available
Explosive properties No information available
VOC Content No information available
Specific Gravity no data available
Partition coefficient: no data available
Vapor pressure no data available
Viscosity No information available
Vapor density No data available
Initial Boiling Point No information available
Evaporation Rate No information available
Decomposition Temperature °C No information available

10. STABILITY AND REACTIVITY

Stability Stable under normal conditions.

Conditions to Avoid Strong acids Oxidizing agents

Hazardous Decomposition Products None under normal use.

11. TOXICOLOGICAL INFORMATION

Caution - substance not yet tested completely

Acute Toxicity

Carcinogenicity There are no known carcinogenic chemicals in this product.

Target Organ Effects None known.

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Skin contact Rinse immediately with plenty of water and seek medical advice.

Inhalation Move to fresh air.

Ingestion Never give anything by mouth to an unconscious person. Clean mouth with water.

5. FIRE-FIGHTING MEASURES

Flammable Properties Not flammable.
Flash point No information available
Suitable Extinguishing Media Dry chemical, CO, alcohol-resistant foam or water spray.
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.

NFPA Health Hazard 0 **Flammability** 0 **Stability** 0 **Physical and chemical hazards**

6. ACCIDENTAL RELEASE MEASURES

Personal precautions Avoid contact with skin, eyes and clothing.

Methods for Containment Prevent further leakage or spillage if safe to do so.

Methods for cleaning up Prevent product from entering drains.

7. HANDLING AND STORAGE

Advice on safe handling Ensure adequate ventilation. Handle in accordance with good industrial hygiene and safety practice.

Technical measures/Storage conditions Keep container tightly closed. Recommended storage temperature 4 °C

8. EXPOSURE CONTROLS/PERSONAL PROTECTION

Exposure Guidelines This product does not contain any hazardous materials with occupational exposure limits established by the region specific regulatory bodies.

Chemical Name	ACGIH TLV	OSHA PEL	NIOSH IDLH
Glycerol 56-81-5	TWA: 10 mg/m ³	TWA: 15 mg/m ³ TWA: 5 mg/m ³ (vacated) TWA: 10 mg/m ³ (vacated) TWA: 5 mg/m ³	

Engineering Measures Showers, eyewash stations, and ventilation systems.

Hygiene measures When using, do not eat, drink or smoke Wear suitable gloves and eye/face protection Wash hands before breaks and at the end of workday Wash hands with water as a precaution Regular cleaning of equipment, work area and clothing is recommended Avoid breathing vapors, mist or gas

Personal Protective Equipment

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12. ECOLOGICAL INFORMATION

Ecotoxicity

The environmental impact of this product has not been fully investigated.

Persistence and degradability No information available.

Bioaccumulation No information available.

Mobility No information available.

Chemical Name	log Pow
Glycerol	-1.76

13. DISPOSAL CONSIDERATIONS

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

14. TRANSPORT INFORMATION

DOT Not regulated

MEX Not regulated

IATA Not regulated

15. REGULATORY INFORMATION

OSHA Regulatory Status

While this material is not considered hazardous by the OSHA Hazard Communication Standard (29 CFR 1910.1200), this MSDS contains valuable information critical to the safe handling and proper use of the product. This MSDS should be retained and available for employees and other users of this product

International Inventories

TSCA -
DSL/NDL -
EINECS/ELINCS -
ENCS -
IECS -
KECL -
PICCS -
AICS -
NZIoC -

U.S. Federal Regulations

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.

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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).

Clean Air Act, Section 112 Hazardous Air Pollutants (HAPs) (see 40 CFR 61)
This product does not contain any substances regulated as hazardous air pollutants (HAPS) under Section 112 of the Clean Air Act Amendments of 1990.

Chemical Name	CAS-No	Weight %	HAPS data	VOC Chemicals	Class 1 Ozone Depleters	Class 2 Ozone Depleters
Glycerol	56-81-5	30 - 60		Group II		

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.

TSCA

Chemical Name	U.S. - TSCA (Toxic Substances Control Act) - Section 8(a) - Chemical-Specific Reporting and Recordkeeping
Glycerol	Partially exempt chemical substance under 40 CFR 710.46(b)(2)

U.S. State Regulations
California Proposition 65
This product does not contain any Proposition 65 chemicals.

U.S. State Right-to-Know Regulations
This product does not contain any substances regulated by state right-to-know regulations.

Chemical Name	Massachusetts	New Jersey	Pennsylvania	Illinois	Rhode Island
Glycerol	X	X	X		X

International Regulations
Mexico - Grade No information available.

Chemical Name	Carcinogen Status	Exposure Limits
Glycerol		Mexico: TWA 10 mg/m ³

Canada
This product has been classified in accordance with the hazard criteria of the Controlled Products Regulations (CPR) and the MSDS contains all the information required by the CPR.
WHMIS Hazard Class
Not Determined

16. OTHER INFORMATION

Revision Date: 2010-08-24
Revision Note: No information available.

Disclaimer
The information provided on this MSDS 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 guide for safe handling, use, processing, storage, transportation, disposal and release and is not to be considered as 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 material or in any process, unless specified in the text.

End of Material Safety Data Sheet