Store at -20°C

#7261

PhosphoPlus® S6 Ribosomal Protein (Ser235/236) In-Cell Duet (ICW Compatible)

☐ Small Kit
(1 x 96 well plate)

☐ Large Kit
(5 x 96 well plates)

New 08/10

Cell Signaling TECHNOLOGY®

Orders ■ 877-616-CELL (2355)
orders@cellsignal.com
Support ■ 877-678-TECH (8324)
info@cellsignal.com
Web ■ www.cellsignal.com

This product is intended for research purposes only. This product is not intended to be used for therapeutic or diagnostic purposes in humans or animals.

Products Included	Product #	Volume	Applicaton	Dilution	Species Cross-Reactivity
Primary Cocktail	5534	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-S6 Ribosomal Protein (Ser235/236)	DyLight® 800	777	794
Total S6 Ribosomal Protein	DyLight® 680	692	712

Description: PhosphoPlus® S6 Ribosomal Protein (Ser235/236) 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-S6 Ribosomal Protein (Ser235/236) detects endogenous levels of ribosomal protein S6 only when phosphorylated at Ser235 and 236. Total S6 Ribosomal Protein detects endogenous levels of total S6 ribosomal protein independent of phosphorylation.

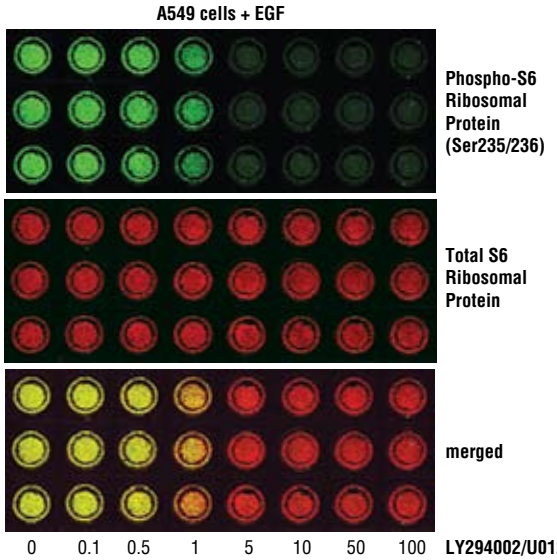
Source/Purification: Monoclonal antibodies are produced by immunizing animals with a synthetic phosphopeptide corresponding to residues surrounding Ser235 and Ser236 of human ribosomal protein S6 or with a GST-S6 ribosomal protein (full length) fusion protein.

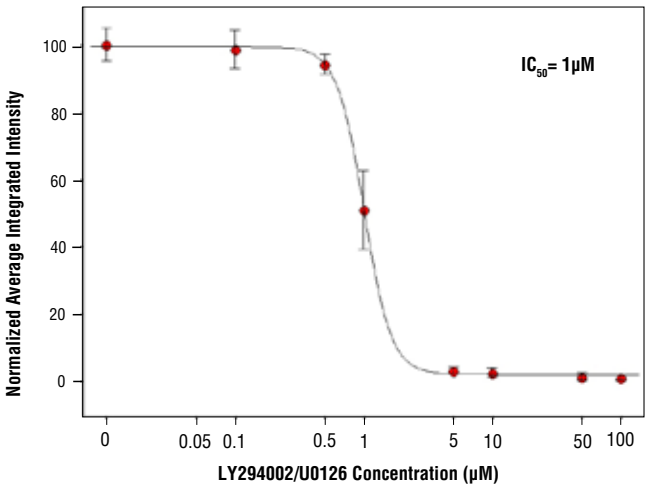
Storage: Supplied in 10 mM sodium HEPES (pH 7.5), 150 mM, NaCl, 100 µg/ml BSA and 50% glycerol. Store at –20°C. *Do not aliquot either cocktail.*

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 A549 cells exposed to varying concentrations of LY294002 (PI3 Kinase Inhibitor) #9901 and U0126 (MEK1/2 Kinase Inhibitor) #9903 for 2 hours, followed by stimulation with hEGF #8916 for 20 minutes. The phosphorylation status of S6 Ribosomal Protein, as well as the total protein expression level, was measured simultaneously using the PhosphoPlus® S6 Ribosomal Protein (Ser235/236) In-Cell Duet (ICW Compatible) #7261. With increasing concentrations of LY294002 and U0126, a significant decrease (~10-fold) in phospho-S6 signal as compared to the hEGF-stimulated control was observed, while total S6 protein levels remained unchanged and were used to normalize the data. When using phospho-S6 as a measurement, the IC_{50} of these compounds was 1 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.

© 2010 Cell Signaling Technology, Inc.

Background: One way that growth factors and mitogens effectively promote sustained cell growth and proliferation is by upregulating mRNA translation (1,2). Growth factors and mitogens induce the activation of p70 S6 kinase and the subsequent phosphorylation of the S6 ribosomal protein. Phosphorylation of S6 ribosomal protein correlates with an increase in translation of mRNA transcripts that contain an oligopyrimidine tract in their 5' untranslated regions (2). These particular mRNA transcripts (5'TOP) encode proteins involved in cell cycle progression as well as ribosomal proteins and elongation factors necessary for translation (2,3). Important S6 ribosomal protein phosphorylation sites include several residues (Ser235, Ser236, Ser240, and Ser244) located within a small, carboxy-terminal region of the S6 protein (4,5).

Background References:

(1) Dufner, A. and Thomas, G. (1999) *Exp. Cell Res.* 253, 100-109.

(2) Peterson, R.T. and Schreiber, S.L. (1998) *Curr. Biol.* 8, R248-R250.

(3) Jefferies, H.B. et al. (1997) *EMBO J.* 16, 3693-3704.

(4) Ferrari, S. et al. (1991) *J. Biol. Chem.* 266, 22770-22775.

(5) Flotow, H. and Thomas, G. (1992) *J. Biol. Chem.* 267, 3074-3078.

© 2010 Cell Signaling Technology, Inc.

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.

© 2010 Cell Signaling Technology, Inc.

Material Safety Data Sheet (MSDS)

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.

Appearance aqueous solution **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	9048-46-8	< 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.

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

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.

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/NDSL	-
EINECS/ELINCS	-
ENCS	-
IECSC	-
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.

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 Depletors	Class 2 Ozone Depletors
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