#7851 Store at -20°C

PathScan® Apoptosis and Proliferation Multiplex IF Kit

☑ 1 Kit
(100 tests)

Cell Signaling TECHNOLOGY®

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

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

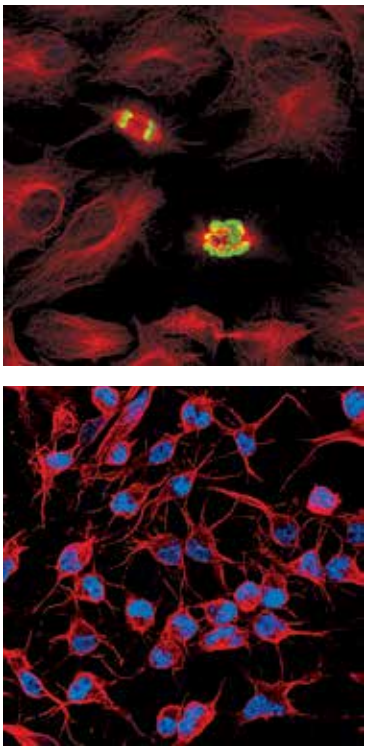
Products Included	Product #	Volume	Applicaton	Dilution	Species Cross-Reactivity
Primary Cocktail	7847	100 µl	IF-IC, IF-P*	1:100	H, (Mk)
Detection Cocktail	7843	100 µl	IF-IC, IF-P*	1:100	N/A

Kit Analytes	Detection Dye	$Ex_{(max)}$ (nm)	$Em_{(max)}$ (nm)
Phospho-Histone H3 (Ser10)	Alexa Fluor® 488	495	519
Cleaved-PARP (Asp214)	Alexa Fluor® 647	650	665
α-Tubulin	Alexa Fluor® 555	555	565

Description: The PathScan® Apoptosis and Proliferation Multiplex IF kit offers a novel method to simultaneously monitor mitotic index and programmed cell death using manual immunofluorescence microscopy, or automated imaging and laser scanning high content platforms. This kit contains a cocktail of three high quality primary antibodies targeted against α-tubulin, phospho-histone H3 (Ser10), and cleaved-PARP (Asp214), as well as a detection cocktail utilizing the Alexa Fluor® series of fluorescent dyes. Antibody and dye pairings have been pre-optimized, and each kit contains enough reagents for 100 assays (based on a working volume of 100 µL/test).

Background: Apoptosis is a regulated physiological process leading to cell death. Initiator caspases cleave and activate downstream effector caspases that in turn cleave cytoskeletal and nuclear proteins such as PARP, α-fodrin, DFF, and lamin A, which induce apoptosis (1). Cleavage of the nuclear poly (ADP-ribose) polymerase PARP occurs between Asp214 and Gly215, which separates the PARP amino-terminal DNA binding domain (24 kDa) from the carboxy-terminal catalytic domain (89 kDa) (2,3). PARP helps to maintain cell viability by playing key roles in many cellular processes, including DNA replication, repair, and recombination. Cleavage of PARP facilitates cellular disassembly and serves as a marker of cells undergoing apoptosis (4). Cell proliferation can be measured by studying the phosphorylation state of histone H3 and microtubule assembly during mitosis. Histone H3 is one of four distinct core histone proteins found in the nucleosome. Phosphorylation of histone H3 at Ser10, Ser28, and Thr11 is tightly correlated with chromosome condensation during both mitosis and meiosis (5-7). Heterodimers composed of α-tubulin and β-tubulin form globular tubulin subunits common to all eukaryotic cells. Tubulin polymers known as microtubules are fundamental cytosolic fibers important in mediating cellular movement, including meiotic/mitotic chromosome alignment, cytoplasmic membrane vesicle transport, and nerve-cell axon migration (8).

Specificity/Sensitivity: α-Tubulin antibody detects endogenous levels of total α-tubulin protein. Phospho-Histone H3 (Ser10) antibody detects endogenous levels of histone H3 only when phosphorylated at Ser10. This antibody does not cross-react with other phosphorylated or acetylated histones. Cleaved-PARP (Asp214) detects endogenous levels of the large fragment (89 kDa) of human PARP1 protein produced by caspase cleavage. This antibody does not recognize full length PARP1 or other PARP isoforms.



Confocal immunofluorescent analysis of HeLa cells, untreated (upper) or treated with Staurosporine #9953 (lower), using the PathScan® Apoptosis and Proliferation Multiplex IF Kit. Red = α-tubulin, green = phospho-Histone H3 (Ser10), and blue = cleaved-PARP (Asp214)

Source/Purification: Monoclonal antibodies are produced by immunizing animals with a synthetic phosphopeptide corresponding to residues surrounding Ser10 of human histone H3, a synthetic peptide corresponding to residues surrounding Asp213 of human PARP, or with full-length chicken α-tubulin purified from brain extracts.

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 either cocktail.*

*IF-P protocol recommended unmasking buffer: Citrate

Background References:

(1) Nicholson, D.W. (1999) *Cell Death Differ* 6, 1028-42.
(2) Lazebnik, Y.A. et al. (1994) *Nature* 371, 346-7.
(3) Nicholson, D.W. et al. (1995) *Nature* 376, 37-43.
(4) Oliver, F.J. et al. (1998) *J Biol Chem* 273, 33533-9.
(5) Hendzel, M.J. et al. (1997) *Chromosoma* 106, 348-60.
(6) Goto, H. et al. (1999) *J Biol Chem* 274, 25543-9.
(7) Preuss, U. et al. (2003) *Nucleic Acids Res* 31, 878-85.
(8) Westermann, S. and Weber, K. (2003) *Nat Rev Mol Cell Biol* 4, 938-47.

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

#7851

PathScan® Multiplex IF Kit Protocol

***IMPORTANT:** Please refer to the **APPLICATIONS** section on the front page of the datasheet to determine if this kit has been validated and approved for use on cultured cell lines (**IF-IC**), paraffin-embedded samples (**IF-P**), or frozen tissue sections (**IF-F**)

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,** 16%, methanol free, Polysciences, Inc. (cat# 18814), use fresh, store opened vials at 4°C in dark, dilute in warm 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 dH2O, mix. Add 0.25 g BSA and mix well. While stirring, add 75 µl Triton X-100.

Reagents specific to IF-P application:

1. Xylene.
2. Ethanol, anhydrous denatured, histological grade, 100% and 95%.
3. **Antigen Unmasking:**
   a. **Citrate:** 10 mM Sodium Citrate Buffer: To prepare 1 L add 2.94 g sodium citrate trisodium salt dihydrate ($C_6H_5Na_3O_7 \bullet 2H_2O$) to 1 L dH_2O. Adjust pH to 6.0.
   b. **EDTA:** 1 mM EDTA: To prepare 1 L add 0.372 g EDTA ($C_{10}H_{14}N_2O_8Na_2 \bullet 2H_2O$) to 1 L dH_2O. Adjust pH to 8.0.

B Specimen Preparation

I. Cultured Cell Lines (IF-IC)

NOTE: Cells should be grown, treated, fixed, and stained directly in multiwell plates, chamber slides, or on coverslips.

1. Aspirate culture medium, and then cover cells to a depth of 2–3 mm with 4% formaldehyde diluted in 1X PBS warmed to 37°C.
   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 (Section C).

II. Paraffin Sections (IF-P)

NOTE: Do not allow slides to dry at any time during this procedure.

Deparaffinization/Rehydration:

1. Incubate sections in three washes of xylene for 5 minutes each.
2. Incubate sections in two washes of 100% ethanol for 10 minutes each.
3. Incubate sections in two washes of 95% ethanol for 10 minutes each.
4. Rinse sections twice in dH_2O for 5 minutes each.

NOTE: Consult product datasheet for specific recommendation for the unmasking solution.

Antigen Unmasking:

1. **For Citrate:** Bring slides to a boil in 10 mM sodium citrate buffer pH 6.0 then maintain at a sub-boiling temperature for 10 minutes. Cool slides on bench top for 30 minutes.
2. **For EDTA:** Bring slides to a boil in 1 mM EDTA pH 8.0 followed by 15 minutes at a sub-boiling temperature. No cooling is necessary.

III. Frozen/Cryostat Sections (IF-F)

NOTE: Fresh frozen/unfixed sections should be fixed immediately in 4% formaldehyde as follows to preserve signaling epitopes.

1. Cover sections with 4% formaldehyde diluted in 1X PBS warmed to 37°C.
   NOTE: Formaldehyde is toxic, use only in fume hood.
2. Allow sections to fix for 15 minutes at room temperature.
3. Rinse slides three times in PBS for 5 minutes each.

C Immunostaining

NOTE: All subsequent incubations should be carried out at room temperature unless otherwise noted in a humid, light-tight box or covered dish/plate to prevent drying and fluorochrome fading.

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. Coverslip slides with Prolong® Gold Antifade Reagent.
10. For best results examine specimens immediately using appropriate excitation wavelengths. For long-term storage, store slides at 4°C protected from light.

MSDS

Material Safety Data Sheet (MSDS) for PathScan® Signaling Nodes Multiplex IF Kit

Cell Signaling TECHNOLOGY®

I. Identification:

Product name: PathScan® Signaling Nodes Multiplex IF Kit
Product Catalog: 8999
CAS#: 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:

This Product is For Research Use Only.
The components of this kit are composed of antibodies in aqueous buffer solution.

Hazardous Ingredient:	Percent (%w/v)	CAS#	EC#
Glycerol	53%	56-81-5	200-289-5
Sodium azide	<0.02%	26628-22-8	247-852-1

III. Hazard Identification:

This product is a kit containing antibodies in aqueous solution.
Emergency Overview of Hazardous ingredient substance : Glycerol (CAS# 56-81-5)
According to OSHA, 29 CFR 1910.1200(d): Irritant. Avoid contact and inhalation.
Target organ: Kidneys.
According to (EC) No1272/2008: Eye Irritation (Category 2)
Not a hazardous substance or mixture according to EC-directives 67/548/EEC or 1999/45/EC.
Caution: This substance has not been thoroughly tested.

IV. First Aid Measures:

Inhalation: Remove to fresh air. If breathing is difficult, get medical attention.
Ingestion: If swallowed, rinse mouth with water provided person is conscious. 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 with 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 4°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. Physical And Chemical Properties:

Physical State:	liquid
Appearance:	colorless
Odor:	odorless
pH:	data not available
Boiling Point:	data not available
Melting Point:	data not available
Volatile Organic Compounds (VOC):	data not available
Solubility (water):	soluble

X. Stability and Reactivity:

Stability: : Stable under recommended conditions.
Conditions to avoid: No data available
Hazardous Decomposition: May form carbon dioxides under fire conditions.
Materials to aviod: Strong oxidizing reagents.

XI. Toxicological Information:

Acute toxicity: No data available.
Skin corrosion/irritation: No data available.
Eye damage/eye irritation: No data available.
Mutagenicity: No data available.
Carcinogenicity: No data available.
IARC: No component of this product present at levels greater than or equal to 0.1% is identified as probable, possible, or confirmed human carcinogen by IARC.
Reproductive toxicity: No data available.
Specific target organ toxicity: No data available.

Potential Health Effects:
Inhalation: May be harmful if inhaled.
Ingestion: May be harmful if swallowed.
Skin: May be harmful if absorbed through skin. May cause skin irritation.
Eyes: May cause skin irritation.

XII. Ecological Information:

Toxicity: No data available.
Persistance and degradability: No data available.
Bioaccumulative potential: No data available.
Mobility in soil: No data available.
PBT and vPvP assessment: No data available.
Other adverse effects: No data available.

XIII. Disposal Considerations:

Dispose of in accordance with federal, state and local environment regulations.

XIV. Transport Information:

D.O.T.: This product is considered to be non-hazardous for transport.
IATA: This product is considered to be non-hazardous for transport.
IMDG: This product is considered to be non-hazardous for transport.

XV. Regulatory Information:

This safety datasheet complies with the requirement of regulations 29 CFR 1910.1200(d) and (EC) No.1907/2006.

XVI. Other Information:

This product 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.