

#7967 Store at -20°C

PathScan® EGF Receptor Activation Multiplex IF Kit



1 Kit
 (100 immunocytochemical stainings)

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Support ■ 877-678-TECH (8324)
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rev. 03/15/11

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	7972	100 µl	IF-IC, IF-P*	1:100	H, Mk, (M)
Detection Cocktail	7973	100 µl	IF-IC, IF-P*	1:100	N/A

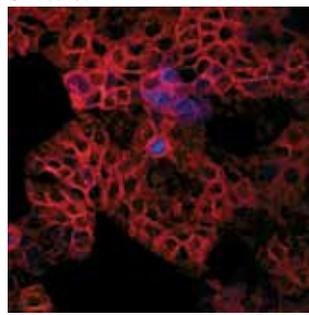
Kit Analytes	Detection Dye	Ex _(max) (nm)	Em _(max) (nm)
EGF Receptor	Alexa Fluor® 555	555	565
Phospho-EGF Receptor (Tyr1068)	Alexa Fluor® 488	495	519
Phospho-p44/42 MAPK (Erk1/2) (Thr202/Tyr204)	Alexa Fluor® 647	650	665

Description: The PathScan® EGF Receptor Activation Multiplex IF Kit offers a novel method to simultaneously monitor the expression, localization, and activation state of EGF receptor, as well as downstream signaling through Erk1/2, 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 total EGF receptor, phospho-EGF receptor (Tyr1068), and phospho-p44/42 MAPK (Erk1/2) (Thr202/Tyr204), 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).

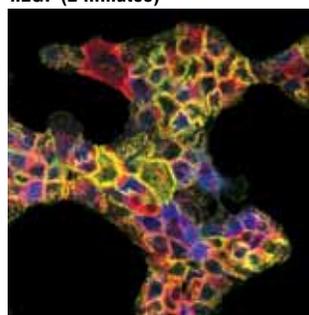
Specificity/Sensitivity: EGF receptor antibody detects endogenous levels of total EGF receptor protein and does not cross-react with other ErbB family members. Phospho-EGF receptor (Tyr1068) antibody detects endogenous EGF receptor only when phosphorylated at Tyr1068. This antibody may cross-react weakly with other tyrosine-phosphorylated proteins. Phospho-p44/42 MAPK (Erk1/2) (Thr202/Tyr204) antibody detects endogenous levels of p44 and p42 MAP kinase (Erk1 and Erk2) when dually phosphorylated at Thr202 and Tyr204 of Erk1 (Thr185 and Tyr187 of Erk2), and singly phosphorylated at Thr202. This antibody does not cross-react with the corresponding phosphorylated residues of either JNK/SAPK or p38 MAP kinases.

Source/Purification: Monoclonal antibodies were produced by immunizing animals with synthetic phosphopeptides corresponding to residues surrounding Thr202/Tyr204 of human p44 MAP kinase and Tyr1068 of human EGF receptor, or with a fusion protein containing the cytoplasmic domain of human EGF receptor.

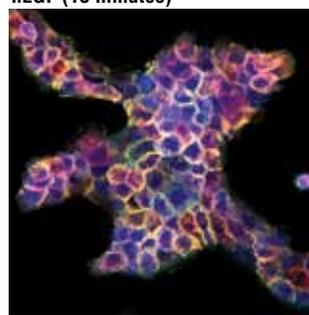
Untreated



hEGF (2 minutes)



hEGF (15 minutes)



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.*

*IF-P protocol recommended unmasking buffer: EDTA

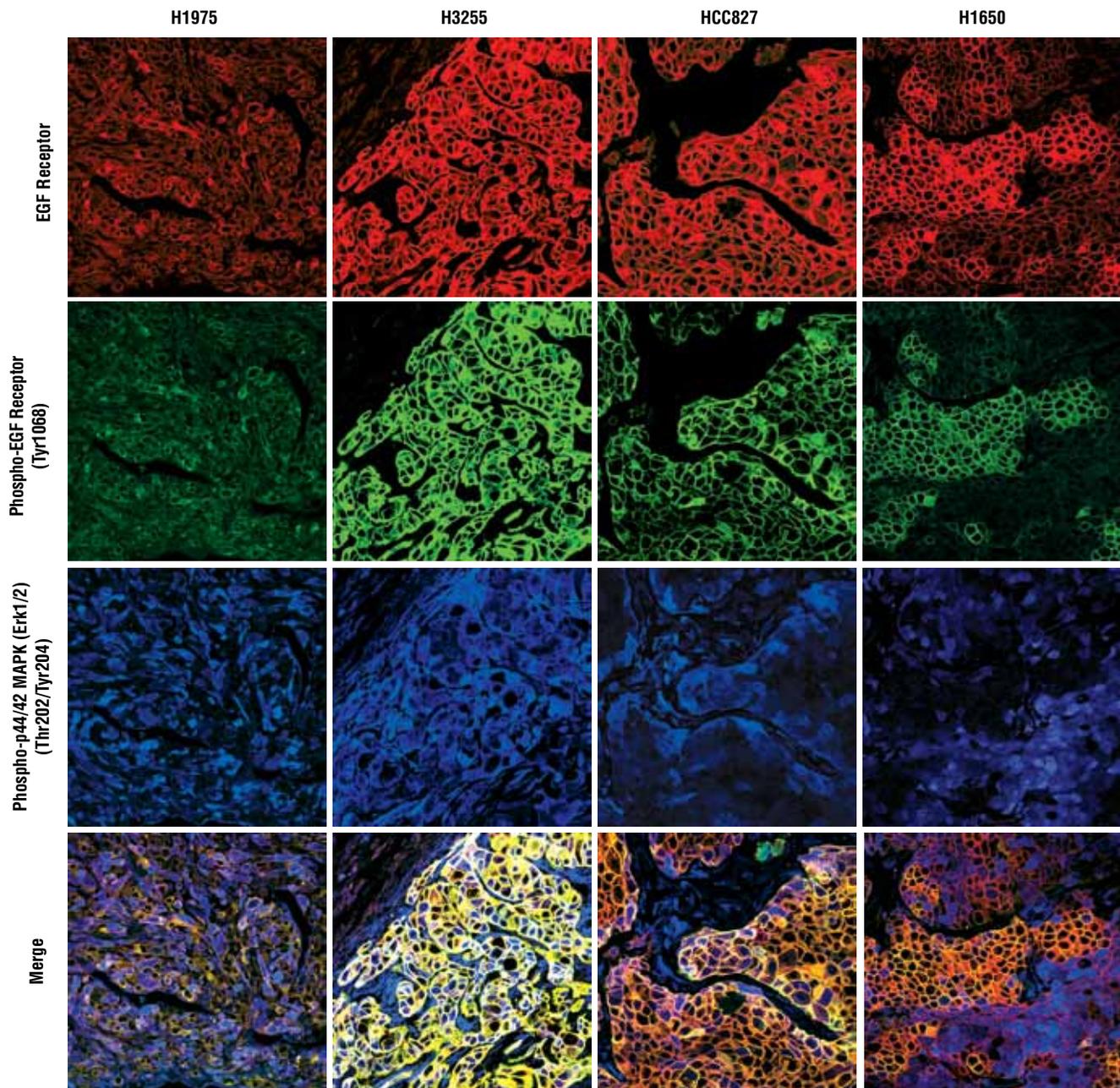
Some kit components are provided under an agreement between Life Technologies Corporation and Cell Signaling Technology, Inc., and the manufacture, use, sale or import of antibody conjugate in this product is subject to one or more US patents and corresponding non-US equivalents, owned or controlled by Life Technologies Corporation or its affiliates. The purchase of this product conveys to the buyer the non-transferable right to use the purchased amount of the product and components of the product only in research conducted by the buyer (whether the buyer is an academic or for-profit entity), for immunocytochemistry, high content screening (HCS) analysis, or flow cytometry applications. Buyer's use of this product or its components (1) in manufacturing; (2) to provide a service, information, or data to an unaffiliated third party for payment; (3) for therapeutic, diagnostic or prophylactic purposes; (4) resale, whether or not such product or its components are resold for use in research; or for any other commercial purpose is prohibited. For information on purchasing a license to this product for purposes other than research, contact Life Technologies Corporation, Cellular Analysis Business Unit, Business Development, 29851 Willow Creek Road, Eugene, OR 97402, Tel: (541) 465-8300. Fax: (541) 335-0354.

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◀ *Confocal immunofluorescent analysis of serum-starved A431 cells, treated with Human Epidermal Growth Factor (hEGF) #8916 (100 ng/ml) as indicated, using PathScan® EGF Receptor Activation Multiplex IF Kit. Red = EGF receptor, green = phospho-EGF receptor (Tyr1068), and blue pseudocolor = phospho-p44/42 MAPK (Erk1/2) (Thr202/Tyr204).*

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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 Ce—C. elegans Hr—horse All—all species expected Species enclosed in parentheses are predicted to react based on 100% homology.



Confocal immunofluorescent analysis of paraffin-embedded H1975, H3255, HCC827 and H1650 xenografts using PathScan® EGF Receptor Activation Multiplex IF Kit. Red = EGF receptor, green = phospho-EGF receptor (Tyr1068), and blue pseudocolor = phospho-p44/42 MAPK (Erk1/2) (Thr202/Tyr204).

Background: The epidermal growth factor receptor (EGFR) is a 170 kDa transmembrane receptor tyrosine kinase that belongs to the HER/ErbB protein family that includes HER2/ErbB2/neu, HER3/ErbB3, and HER4/ErbB4 (1,2). Ligand binding results in receptor homo- and heterodimerization which stimulates its intrinsic tyrosine kinase activity. Autophosphorylation at residues Tyr992, Tyr1045, Tyr1068, Tyr1148, and Tyr1173, as well as c-Src mediated phosphorylation at Tyr845 and Tyr1101 promote docking of SH2 domain-bearing signaling proteins resulting in cellular responses to EGFR activation (2-6). For example, phosphorylation of Tyr1068 facilitates recruitment of Grb2 which, via association with Sos, stimulates the GTP binding activity of Ras, leading to the activation of MAP kinase and other signaling cascades (7). Following activation, EGFR is rapidly endocytosed and either recycled back to the plasma membrane or targeted for lysosomal degradation (8). Dysregulation of EGFR signaling through activating

mutations or gene amplification has been implicated in the pathogenesis of many human malignancies, leading to intense clinical study of this pathway (9-13).

Background References:

- (1) Hackel, P.O. et al. (1999) *Curr Opin Cell Biol* 11, 184-9.
- (2) Zwick, E. et al. (1999) *Trends Pharmacol Sci* 20, 408-12.
- (3) Cooper, J.A. and Howell, B. (1993) *Cell* 73, 1051-4.
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- (5) Emler, D.R. et al. (1997) *J Biol Chem* 272, 4079-86.

- (6) Levkowitz, G. et al. (1999) *Mol Cell* 4, 1029-40.
- (7) Rojas, M. et al. (1996) *J Biol Chem* 271, 27456-61.
- (8) Lai, W.H. et al. (1989) *J Cell Biol* 109, 2741-9.
- (9) Yarden, Y. and Sliwkowski, M.X. (2001) *Nat Rev Mol Cell Biol* 2, 127-37.
- (10) Press, M.F. and Lenz, H.J. (2007) *Drugs* 67, 2045-75.
- (11) Baselga, J. (2002) *Oncologist* 7 Suppl 4, 2-8.
- (12) Foon, K.A. et al. (2004) *Int J Radiat Oncol Biol Phys* 58, 984-90.
- (13) Sridhar, S.S. et al. (2003) *Lancet Oncol* 4, 397-406.

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.

- 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.
- Formaldehyde,** 16%, methanol free, Polysciences, Inc. (cat# 18814), use fresh, store opened vials at 4°C in dark, dilute in warm PBS for use.
- 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.
- 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.

Reagents specific to IF-P application:

- Xylene.
- Ethanol, anhydrous denatured, histological grade, 100% and 95%.
- Antigen Unmasking:**
 - Citrate:** 10 mM Sodium Citrate Buffer: To prepare 1 L add 2.94 g sodium citrate trisodium salt dihydrate ($\text{C}_6\text{H}_5\text{Na}_3\text{O}_7 \cdot 2\text{H}_2\text{O}$) to 1 L dH_2O . Adjust pH to 6.0.
 - EDTA:** 1 mM EDTA: To prepare 1 L add 0.372 g EDTA ($\text{C}_{10}\text{H}_{14}\text{N}_2\text{O}_8\text{Na}_2 \cdot 2\text{H}_2\text{O}$) 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.

- 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.
- Allow cells to fix for 15 minutes at room temperature.
- Aspirate fixative, rinse three times in PBS for 5 minutes each.
- 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:

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

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

*Antigen Unmasking:

- 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.
- 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.

- Cover sections with 4% formaldehyde diluted in 1X PBS warmed to 37°C.
NOTE: Formaldehyde is toxic, use only in fume hood.
- Allow sections to fix for 15 minutes at room temperature.
- 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.

- Block specimen in Blocking Buffer for 60 minutes.
- While blocking, prepare primary cocktail by diluting as indicated on datasheet in Antibody Dilution Buffer.
- Aspirate blocking solution, apply diluted primary cocktail.
- Incubate overnight at 4°C.
- Rinse three times in PBS for 5 minutes each.
- Prepare detection cocktail by diluting as indicated on datasheet in Antibody Dilution Buffer.
- Incubate 1–2 hours at room temperature in the dark.
- Rinse three times in PBS for 5 minutes each.
- Coverslip slides with Prolong® Gold Antifade Reagent.
- For best results examine specimens immediately using appropriate excitation wavelengths. For long-term storage, store slides at 4°C protected from light.

Material Safety Data Sheet (MSDS) for PathScan® Multiplex IF Kits

I. Identification:

Product name: PathScan® Multiplex IF Kits

Product Catalog: 8999, 7967

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 avoid: 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.

Persistence 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.