Store at -20°C

#7771

PathScan® EMT Duplex IF Kit

☑ 1 Kit
(100 tests)

Cell Signaling TECHNOLOGY®

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orders@cellsignal.com
Support ■ 877-678-TECH (8324)
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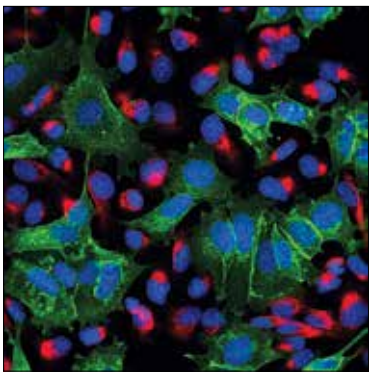
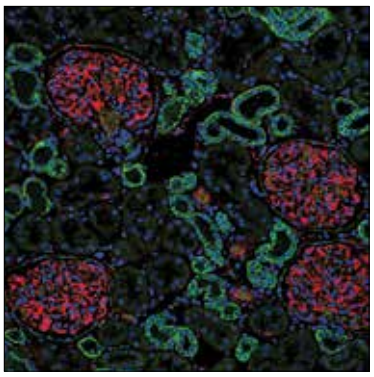
For Research Use Only. Not For Use In Diagnostic Procedures.

Products Included	Product #	Volume	Applicaton	Dilution	Species Cross-Reactivity
PathScan® EMT Duplex IF Kit Primary Antibody Cocktail	7783	100 µl	IF-IC, IF-P*	1:100	H, (M)
PathScan® Duplex IF Kit Detection Cocktail I	7832	100 µl	IF-IC, IF-P*	1:100	n/a

Kit Analytes	Detection Dye	$Ex_{(max)}$ (nm)	$Em_{(max)}$ (nm)
E-cadherin	Alexa Fluor® 488	495	519
Vimentin	Alexa Fluor® 555	555	565



Confocal immunofluorescent analysis of cocultured MCF7 and MDA-MB-231 cells using PathScan® EMT Duplex IF Kit. Green = E-cadherin, red = vimentin, and blue pseudocolor = DRAQ5® #4084 (fluorescent DNA dye).



Confocal immunofluorescent analysis of paraffin-embedded human kidney using PathScan® EMT Duplex IF Kit. Green = E-cadherin, red = vimentin, and blue pseudocolor = DRAQ5® #4084 (fluorescent DNA dye).

Description: The PathScan® EMT Duplex IF Kit offers a novel method to simultaneously monitor cells of epithelial or mesenchymal origin, as well as those undergoing an epithelial-mesenchymal transition (EMT) using manual immunofluorescence microscopy or automated imaging and laser scanning high content platforms. This kit contains a cocktail of two high quality primary antibodies targeted against vimentin and E-cadherin, 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: Epithelial-mesenchymal transition (EMT) refers to a biological process in which cells undergo a series of biochemical changes that induce a morphological transformation from an epithelial, polarized, adhesive state to an irregular, elongated, mesenchymal phenotype that enables migratory capacity (1,2). EMTs are classified into three subtypes: those involved in implantation, embryogenesis, and organ development; those associated with inflammation and fibrosis; and those involved in invasion and metastasis (1). Molecular changes that are associated with cells during this transformation include the loss of E-cadherin and gain of vimentin expression, hallmark epithelial and mesenchymal markers, respectively (3-5). Numerous studies have established that EMT is an essential step in cancer metastasis (5-7). E-cadherin is regarded as an active suppressor of invasion and tumorigenesis (8). In response to extracellular stimuli, vimentin coordinates various signaling pathways to induce spatial reorganization and structural changes (9), reminiscent of the EMT phenotype observed in motile cells involved in invasion and metastasis (6).

Specificity/Sensitivity: Vimentin mAb detects endogenous levels of total vimentin protein. E-cadherin mAb detects endogenous levels of total E-cadherin protein and does not cross react with related family members such as N-cadherin.

Source/Purification: Monoclonal antibodies were produced by immunizing animals with a synthetic peptide corresponding to residues surrounding Arg45 of human vimentin protein or residues surrounding Pro780 of human E-cadherin protein.

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 the antibody.*

***IF-P recommended unmasking buffer: Citrate**

Background References:

(1) Kalluri, R. and Weinberg, R.A. (2009) *J Clin Invest* 119, 1420-8.

(2) Lee, J.M. et al. (2006) *J Cell Biol* 172, 973-81.

(3) Yan, W. et al. (2010) *J Biol Chem* 285, 14042-51.

(4) Sethi, S. et al. (2011) *Transl Oncol* 4, 222-6.

(5) Kim, J.H. et al. (2007) *J Korean Med Sci* 22, 898-904.

(6) Emadi Baygi, M. et al. (2010) *Cell Biol Toxicol* 26, 553-67.

(7) Sethi, S. et al. (2010) *Am J Transl Res* 3, 90-9.

(8) Wheelock, M.J. and Johnson, K.R. (2003) *Annu Rev Cell Dev Biol* 19, 207-35.

(9) Helfand, B.T. et al. (2004) *J Cell Sci* 117, 133-41.

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.

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PathScan® Duplex IF Kit 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,** 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 1:100 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 1:100 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® EMT Duplex IF Kit

Cell Signaling TECHNOLOGY®

I. Identification:

Product name: PathScan® EMT Duplex IF Kit
Product Catalog: 7771
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.