PathScan® Total Met Sandwich ELISA Kit

Store at 4°C

#7242

☑ 1 Kit
(96 assays)

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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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.

Species Cross-Reactivity: H

Introduction: CST's PathScan® Total Met Sandwich ELISA Kit is a solid phase sandwich enzyme-linked immunosorbent assay (ELISA) that detects endogenous levels of total Met protein. A Met Mouse mAb #3127* has been coated onto the microwells. After incubation with cell lysates, both phospho- and nonphospho-Met proteins are captured by the coated antibody. Following extensive washing, Met Rabbit Antibody #3123* is added to detect both the captured phospho- and nonphospho-Met protein. Anti-rabbit IgG, HRP-linked Antibody #7074* is then used to recognize the bound detection antibody. HRP substrate, TMB, is added to develop color. The magnitude of optical density for this developed color is proportional to the quantity of total Met protein.

* Antibodies in kit are custom formulations specific to kit.*

Please visit www.cellsignal.com for a complete listing of recommended companion products.

Specificity/Sensitivity: CST's PathScan® Total Met Sandwich ELISA Kit #7242 detects endogenous levels of total Met protein. As shown in Figure 1, both phospho- and nonphospho-Met proteins from untreated and HGF-treated A431 cell lysates are detected by this kit. In Figure 3, Western blot analysis of protein captured in the Met Mouse mAb #3127 coated microwell shows a single band corresponding to the Met protein.

Background: Met, a high affinity tyrosine kinase receptor for hepatocyte growth factor (HGF, also known as scatter factor) is a disulfide-linked heterodimer made of 45 kDa α- and 145 kDa β-subunits (1,2). The α-subunit and the amino-terminal region of the β-subunit form the extracellular domain. The remainder of the β-chain spans the plasma membrane and contains a cytoplasmic region with tyrosine kinase activity. Interaction of Met with HGF results in autophosphorylation at multiple tyrosines, which recruit several downstream signaling components, including Gab1, c-Cbl, and PI3 kinase (3). These fundamental events are important for all of the biological functions involving Met kinase activity. The addition of a phosphate at cytoplasmic Tyr1003 is essential for Met protein ubiquitination and degradation (4). Phosphorylation at Tyr1234/1235 in the Met kinase domain is critical for kinase activation. Phosphorylation at Tyr1349 in the Met cytoplasmic domain provides a direct binding site for Gab1 (5). Altered Met levels and/or tyrosine kinase activities are found in several types of tumors, including renal, colon, and breast. Thus, Met is an attractive cancer therapeutic and diagnostic target (6,7).

Products Included	Volume	Solution Color
Met Mouse mAb coated microwells*	96 tests	
Met Detection Ab	11 ml	green
Anti-rabbit IgG HRP-Linked Ab	11 ml	red
TMB Substrate	11 ml	colorless
STOP Solution	11 ml	colorless
Sealing Tape	2 sheets	
20X Wash Buffer	25 ml	colorless
Sample Diluent	25 ml	blue
10X Cell Lysis Buffer #9803**	15 ml	yellowish

* 12 8-well modules -Each module is designed to break apart for 8 tests.
**Kit should be stored at 4°C with the exception of 10X Cell Lysis Buffer, which is stored at –20°C (packaged separately).

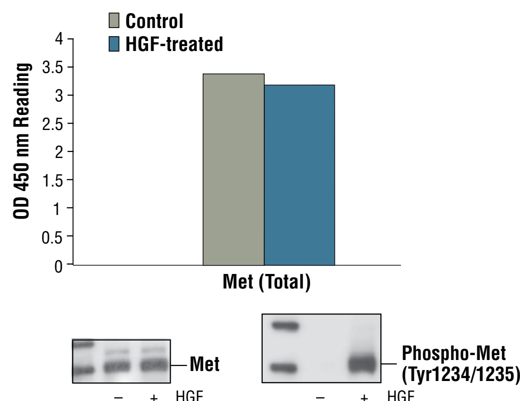

Figure 1: Non-phospho and phospho Met proteins from untreated and HGF-treated A431 cells can be detected by PathScan® Total Met Sandwich ELISA kit #7242 with similar optical density readings. OD 450 readings are shown in the top figure, while the corresponding Western blots using Met Mouse mAb #3127 (left panel) or Met (Tyr1234/1235) Rabbit mAb #3129 (right panel), are shown in the bottom figure.

Background References:

(1) Cooper, C.S. et al. (1984) *Nature* 311, 29-33.
(2) Bottaro, D.P. et al. (1991) *Science* 251, 802-4.
(3) Bardelli, A. et al. (1997) *Oncogene* 15, 3103-11.
(4) Taher, T.E. et al. (2002) *J Immunol* 169, 3793-800.
(5) Schaeper, U. et al. (2000) *J Cell Biol* 149, 1419-32.
(6) Schaeper, U. et al. (2000) *J Cell Biol* 149, 1419-32.
(7) Sattler, M. and Salgia, R. (2009) *Update Cancer Ther* 3, 109-118.

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Applications Key: **W**—Western **IP**—Immunoprecipitation **IHC**—Immunohistochemistry **IC**—Immunocytochemistry **IF**—Immunofluorescence **F**—Flow cytometry **E**—ELISA **D**—DELFIA®

Species Cross-Reactivity Key: **H**—human **M**—mouse **R**—rat **Hm**—hamster **Mk**—monkey **Mi**—mink **C**—chicken **X**—Xenopus **Z**—zebra fish **B**—bovine **All**—all species expected
Species enclosed in parentheses are predicted to react based on 100% sequence homology.

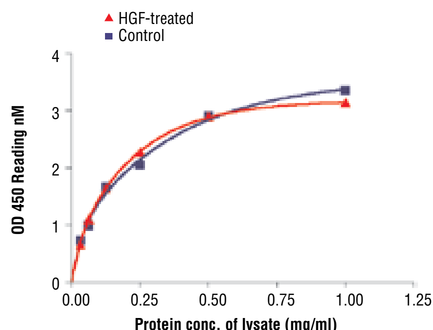

Figure 2: The relationship between protein concentration of lysates from untreated and HGF-treated A431 cells and kit assay optical density readings is shown. After starvation, A431 cells (85% confluence) were treated with HGF (40 ng/ml) for 5 min at 37°C, and then lysed.

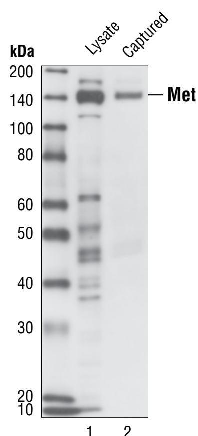

Figure 3: Kit specificity demonstrated by Western blot analysis of the ELISA-well captured protein is shown. Lysates were prepared from human A431 cells and incubated in wells coated with capture antibody #3127. Wells were then washed, and captured protein was solubilized in SDS gel loading buffer. A431 lysate (lane 1) and captured protein (lane 2) were analyzed by Western blot using Met antibody #3123. A single band corresponding to the Met protein is detected in the captured material (lane 2).

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Sandwich ELISA Protocol

A Reagent Preparation

1. Bring all microwell strips to room temperature before use.
2. Prepare 1X Wash Buffer by diluting 20X wash buffer (included in each Pathscan® Sandwich ELISA Kit) in Milli-Q or equivalently purified water.
3. **1X Cell Lysis Buffer from CST #9803:** 20 mM Tris (pH 7.5), 150 mM NaCl, 1 mM ethylene diamine tetraacetate (EDTA), 1 mM ethylene glycol-bis(2-aminoethyl)-N,N,N',N'-tetraacetic acid (EGTA), 1% Triton X-100, 2.5 mM sodium pyrophosphate, 1 mM β-glycerophosphate, 1 mM Na_3VO_4, 1µg/ml leupeptin. This buffer can be stored at 4°C for short-term use (1–2 weeks).

B Preparing Cell Lysates

1. Aspirate media. Treat cells by adding fresh media containing regulator for desired time.
2. To harvest cells under nondenaturing conditions, remove media and rinse cells once with ice-cold PBS.
3. Remove PBS and add 0.5 ml ice-cold 1X Cell Lysis Buffer plus 1 mM phenyl-methylsulfonyl fluoride (PMSF) to each plate (10 cm^2) and incubate the plate on ice for 5 minutes.
4. Scrape cells off the plate and transfer to an appropriate tube. Keep on ice.
5. Sonicate lysates on ice.
6. Microcentrifuge for 10 minutes at 4°C and transfer the supernatant to a new tube. The supernatant is the cell lysate. Store at –80°C in single-use aliquots.

C Test Procedure

1. After the microwell strips have reached room temperature, break off the required number of microwells. Place the microwells in the strip holder. Unused microwells must be resealed and stored at 4°C immediately.
2. Add 100 µl of Sample Diluent (supplied in each Pathscan® Sandwich ELISA Kit, blue color) to a microcentrifuge tube. Transfer 100 µl of cell lysate into the tube and vortex for a few seconds. (Sample applied to the well can be diluted 1:1 when the suggested cell lysis buffer is used for cell extraction.) Individual data sheets for each kit provide information regarding an appropriate dilution factor for lysates and kit assay results.
3. Add 100 µl of each diluted cell lysate to the appropriate well. Seal with tape and press firmly onto top of microwells. Incubate the plate for 2 hours at 37°C. Alternatively, the plate can be incubated overnight at 4°C, which gives the best detection of target protein.
4. Gently remove the tape and wash wells:
   a. Discard plate contents into a receptacle.
   b. Wash 4 times with 1X Wash Buffer, 200 µl each time for each well.
   c. For each wash, strike plates on fresh towels hard enough to remove the residual solution in each well, but do not allow wells to completely dry at any time.
   d. Clean the underside of all wells with a lint-free tissue.
5. Add 100 µl of Detection Antibody (green color) to each well. Seal with tape and incubate the plate for 1 hour at 37°C.
6. Repeat wash procedure as in Step C4.
7. Add 100 µl of HRP-Linked secondary antibody (red color) to each well. Seal with tape and incubate the plate for 30 minutes at 37°C.
8. Repeat wash procedure as in Step C4.
9. Add 100 µl of TMB Substrate to each well. Seal with tape and incubate the plate for 10 minutes at 37°C or 30 minutes at 25°C.
10. Add 100 µl of STOP Solution to each well. Shake gently for a few seconds.

NOTE: Initial color of positive reaction is blue, which changes to yellow upon addition of STOP Solution.

11. Read results.
    a. Visual Determination — Read within 30 minutes after adding STOP Solution.
    b. Spectrophotometric Determination — Wipe underside of wells with a lint-free tissue. Read absorbance at 450 nm within 30 minutes after adding STOP Solution.

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