

PathScan® Phospho-Ron (panTyr) Sandwich ELISA Kit

✓ 1 Kit
(96 assays)



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

Entrez-Gene ID #4486
UniProt ID #Q04912

Species Cross-Reactivity: H

Description: PathScan® Phospho-Ron (panTyr) Sandwich ELISA Kit is a solid phase sandwich enzyme-linked immunosorbent assay (ELISA) that detects endogenous levels of tyrosine-phosphorylated Ron proteins. A Ron Rabbit mAb has been coated onto the microwells. After incubation with cell lysates, Ron protein (phospho and nonphospho) is captured by the coated antibody. Following extensive washing, Phospho-Tyrosine Mouse Detection Antibody is added to detect captured tyrosine-phosphorylated Ron protein. Anti-mouse IgG, HRP-linked Antibody is then used to recognize the bound detection antibody. HRP substrate, TMB, is added to develop color. The magnitude of absorbance for the developed color is proportional to the quantity of tyrosine-phosphorylated Ron protein.

Antibodies in kit are custom formulations specific to kit.

Specificity/Sensitivity: PathScan® Phospho-Ron (panTyr) Sandwich ELISA Kit #12737 detects endogenous levels of Ron protein when phosphorylated at Tyr residues in human cells, as shown in Figure 1. The kit sensitivity is shown in Figure 2. This kit detects proteins from the indicated species, as determined through in-house testing, but may also detect homologous proteins from other species.

Background: Ron is a member of the Met protooncogene family of receptor tyrosine kinases, which also includes Stk, c-Met, and c-Sea. The functional Ron is a heterodimer composed of a 40 kDa α chain and a 150 kDa β chain. Ron is initially synthesized in the cells as a single-chain, pro-Ron precursor that is cleaved into the two active chains. The α chain is completely extracellular, whereas the β chain traverses the cell membrane and contains the intracellular tyrosine kinase and regulatory elements (1,2). Ron mediates multiple signaling cascades that involve cell motility, adhesion, proliferation, and apoptosis. The signaling pathways activated downstream of Ron include the ras/mitogen-activated protein kinase (MAPK), phosphatidylinositol-3 kinase (PI3K)/Akt, and focal adhesion kinase (FAK) pathways. Ron activation can also significantly increase c-Src activity, a signaling intermediate involved in cell cycle progression, motility, angiogenesis and survival (3,4). The function of Ron has been shown to be important for embryological development as well as implicated in the progression and metastasis of tumors (5).

Products Included	Volume	Color
Ron Rabbit mAb Coated Microwells*	96 tests	
Phospho-Tyrosine Mouse Detection mAb	1 each	Green (Lyophilized)
Anti-mouse IgG, HRP-linked Antibody	1 each	Red (Lyophilized)
Detection Antibody Diluent	11 ml	Green
HRP Diluent	11 ml	Red
TMB Substrate #7004	11 ml	Colorless
STOP Solution #7002	11 ml	Colorless
Sealing Tape	2 sheets	
20X ELISA Wash Buffer	25 ml	Colorless
ELISA 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).

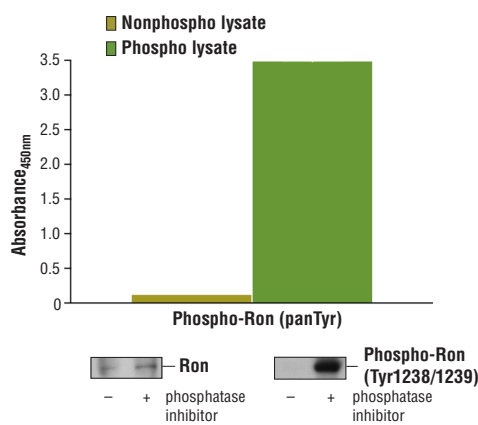


Figure 1. Constitutive phosphorylation of Ron in HCC827 cells lysed in the presence of phosphatase inhibitors* (phospho lysate) is detected by PathScan® Phospho-Ron (panTyr) Sandwich ELISA Kit. In contrast, a low level of phospho-Ron protein is detected in HCC827 cells lysed in the absence of phosphatase inhibitors* (nonphospho lysate). Absorbance at 450 nm is shown in the top figure while corresponding western blots using Ron (81H9) Rabbit mAb #2654 (left) and a Phospho-Ron (Tyr1238/1239) antibody (right) are shown in the bottom figure. *Phosphatase inhibitors include sodium pyrophosphate, β -glycerophosphate, and sodium orthovanadate.

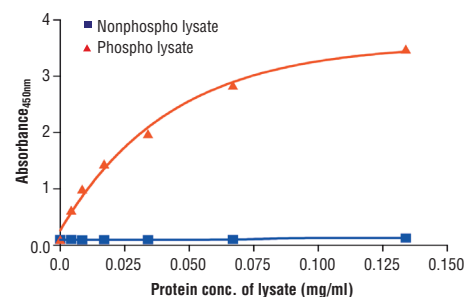


Figure 2. The relationship between protein concentration of phospho or nonphospho lysates and the absorbance at 450 nm is shown. Unstarved HCC827 cells were cultured (85% confluence) and lysed with or without addition of phosphatase inhibitors to the lysis buffer (phospho or nonphospho lysate, respectively).

Background References:

- (1) Ronsin, C. et al. (1993) *Oncogene* 8, 1195-1202.
- (2) Gaudino, G. et al. (1994) *EMBO J.* 13, 3524-3532.
- (3) Wang, M.H. et al. (1996) *Oncogene* 13, 2167-2175.
- (4) Danilkovitch-Miagkova, A. (2003) *Curr. Cancer Drug Targets* 3, 31-40.
- (5) Leonard, E.J. (1997) *Ciba Found. Symp.* 212, 183-191; discussion 192-197.

U.S. Patent No. 5,675,063

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.

PathScan® Sandwich ELISA Protocol (for kits with Lyophilized Antibodies)

A Solutions and Reagents

NOTE: Prepare solutions with purified water.

1. **Microwell strips:** Bring all to room temperature before use.
2. **Detection Antibody:** Supplied lyophilized as a green colored cake or powder. Add 1.0 ml of Detection Antibody Diluent (green solution) to yield a concentrated stock solution. Incubate at room temperature for 5 min with occasional gentle mixing to fully reconstitute. To make the final working solution, add the full 1.0 ml volume of reconstituted Detection Antibody to 10.0 ml of Detection Antibody Diluent in a clean tube and gently mix. Unused working solution may be stored for 4 weeks at 4°C.
3. **HRP-Linked Antibody*:** Supplied lyophilized as a red colored cake or powder. Add 1.0 ml of HRP Diluent (red solution) to yield a concentrated stock solution. Incubate at room temperature for 5 min with occasional gentle mixing to fully reconstitute. To make the final working solution, add the full 1.0 ml volume of reconstituted HRP-Linked Antibody to 10.0 ml of HRP Diluent in a clean tube and gently mix. Unused working solution may be stored for 4 weeks at 4°C.
4. **Detection Antibody Diluent:** Green colored diluent for reconstitution and dilution of the detection antibody (11 ml provided).
5. **HRP Diluent:** Red colored diluent for reconstitution and dilution of the HRP-Linked Antibody (11 ml provided).
6. **Sample Diluent:** Blue colored diluent provided for dilution of cell lysates.
7. **1X Wash Buffer:** Prepare by diluting 20X Wash Buffer (included in each PathScan® Sandwich ELISA Kit) in purified water.
8. **Cell Lysis Buffer:** 10X Cell Lysis Buffer #9803: This buffer can be stored at 4°C for short-term use (1–2 weeks). Recommended: Add 1 mM phenylmethylsulfonyl fluoride (PMSF) immediately before use.
9. **TMB Substrate** (#7004).
10. **STOP Solution** (#7002).

**Note: Some PathScan® ELISA Kits may include HRP-Linked Streptavidin in place of HRP-Linked Antibody.*

B Preparing Cell Lysates

For adherent cells.

1. Aspirate media when the culture reaches 80–90% confluence. Treat cells by adding fresh media containing regulator for desired time.
2. Remove media and rinse cells once with ice-cold 1X PBS.
3. Remove PBS and add 0.5 ml ice-cold 1X Cell Lysis Buffer plus 1 mM PMSF to each plate (10 cm diameter) and incubate the plate on ice for 5 min.
4. Scrape cells off the plate and transfer to an appropriate tube. Keep on ice.
5. Sonicate lysates on ice.
6. Microcentrifuge for 10 min (14,000 rpm) 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.

For suspension cells

1. Remove media by low speed centrifugation (~1200 rpm) when the culture reaches 0.5–1.0 × 10⁶ viable cells/ml. Treat cells by adding fresh media containing regulator for desired time.
2. Collect cells by low speed centrifugation (~1200 rpm) and wash once with 5–10 ml ice-cold 1X PBS.
3. Cells harvested from 50 ml of growth media can be lysed in 2.0 ml of 1X Cell Lysis Buffer plus 1 mM PMSF.
4. Sonicate lysates on ice.
5. Microcentrifuge for 10 min (14,000 rpm) 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. Cell lysates can be undiluted or diluted with Sample Diluent (supplied in each PathScan® Sandwich ELISA Kit, blue color). Individual datasheets for each kit provide a sensitivity curve that serves as a reference for selection of an appropriate starting lysate concentration. The sensitivity curve shows typical kit assay results across a range of lysate concentration points.
3. Add 100 µl of each undiluted or diluted cell lysate to the appropriate well. Seal with tape and press firmly onto top of microwells. Incubate the plate for 2 hr at 37°C. Alternatively, the plate can be incubated overnight at 4°C.
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 reconstituted Detection Antibody (green color) to each well (refer to Section A, Step 2). Seal with tape and incubate the plate at 37°C for 1 hr.
6. Repeat wash procedure (Section C, Step 4).
7. Add 100 µl of reconstituted HRP-Linked secondary antibody (red color) to each well (refer to Section A, Step 3). Seal with tape and incubate the plate for 30 min at 37°C.
8. Repeat wash procedure (Section C, Step 4).
9. Add 100 µl of TMB Substrate to each well. Seal with tape and incubate the plate for 10 min at 37°C or 30 min 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 min after adding STOP Solution.
 - b. **Spectrophotometric Determination:** Wipe underside of wells with a lint-free tissue. Read absorbance at 450 nm within 30 min after adding STOP Solution.