Store at 4°C

#7032C

PathScan® Total Ret Sandwich ELISA Kit

☑ 1 Kit
(96 assays)

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

Entrez-Gene ID #5979
UniProt ID #P07949

Species Cross-Reactivity: H

Description: The PathScan® Total Ret Sandwich ELISA Kit is a solid phase sandwich enzyme-linked immunosorbent assay (ELISA) that detects endogenous levels of total Ret protein. A Ret rabbit mAb has been coated on the microwells. After incubation with cell lysates, Ret protein (phospho and nonphospho) is captured by the coated antibody. Following extensive washing, a Ret mouse mAb is added to detect captured Ret 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 the absorbance for this developed color is proportional to the quantity of total Ret protein.

Specificity/Sensitivity: PathScan® Total Ret Sandwich ELISA Kit #7032 detects endogenous levels of total Ret protein in human cells, as shown in Figure 1. The kit sensitivity is shown in Figure 2.

Background: The Ret proto-oncogene (c-Ret) is a receptor tyrosine kinase that functions as a multicompetent receptor complex in conjunction with other membrane-bound ligand-binding GDNF family receptors (1). Ligands that bind the Ret receptor include the glial cell line-derived neurotropic factor (GDNF) and its congeners neurturin, persephin and artemin (2-4). Alterations in the corresponding Ret gene are associated with diseases including papillary thyroid carcinoma, multiple endocrine neoplasia (type 2A and 2B), familial medullary thyroid carcinoma and a congenital developmental disorder known as Hirschsprung's disease (1,3). The Tyr905 residue located in the Ret kinase domain plays a crucial role in Ret catalytic and biological activity. Substitution of Phe for Tyr905 dramatically inhibits Ret autophosphorylation activity (5).

Background References:

(1) Airaksinen, M.S. et al. (1999) *Mol. Cell. Neurosci.* 13, 313-325.

(2) Takahashi, M. et al. (1989) *Oncogene* 4, 805-806.

(3) Manie, S. et al. (2001) *Trends Genet.* 17, 580-589.

(4) Tallini, G. and Asa, S. (2001) *Adv. Anat. Pathol.* 8, 345-354.

(5) Iwashita, T. et al. (1999) *Oncogene* 18, 3919-3922.

Products Included	Volume	Color
Ret Rabbit mAb Coated Microwells*	96 tests	
Ret 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).

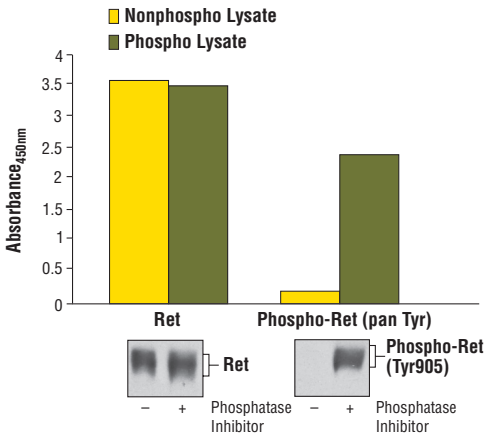

Figure 1: Constitutive phosphorylation of Ret in TT cells lysed in the presence of phosphatase inhibitors (phospho lysate) is detected by PathScan® Phospho-Ret (panTyr) Sandwich ELISA Kit #7034 (upper, right). In contrast, a low level of phospho-Ret protein is detected in TT cells lysed in the absence of phosphatase inhibitors* (nonphospho lysate). Similar levels of total Ret protein from both nonphospho or phospho lysates are detected by PathScan® Total Ret Sandwich ELISA Kit #7032 (upper, left). Absorbance at 450 nm is shown in the top figure while corresponding western blots using a Phospho-Ret (Tyr905) Rabbit Antibody #3221 (right) or a total Ret (C31B4) Rabbit mAb #3223 (left) are shown in the bottom figure. *Phosphatase inhibitors include sodium pyrophosphate, β-glycerophosphate and Na_3VO_4.*

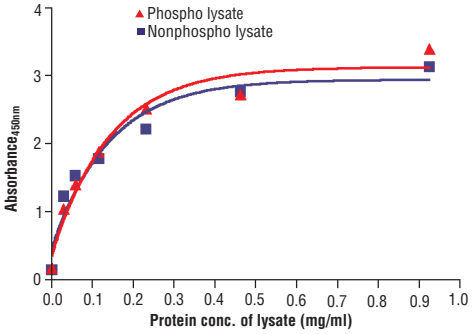

Figure 2: The relationship between protein concentration of phospho or nonphospho lysates and the absorbance at 450 nm is shown. TT cells were cultured (85% confluence) and lysed with or without the addition of phosphatase inhibitor to the lysis buffer (phospho or nonphospho lysate).

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.

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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 x 10^6 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.