

# PathScan® Total Ret Sandwich ELISA Kit

✓ 1 Kit  
(96 assays)

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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.

Entrez-Gene ID #5979  
Swiss-Prot Acc. #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 neurotrophic 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	Solution Color
Ret Rabbit mAb Coated Microwells*	96 tests	
Ret Mouse Detection mAb	11 ml	Green
Anti-mouse IgG, HRP-linked Antibody	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).

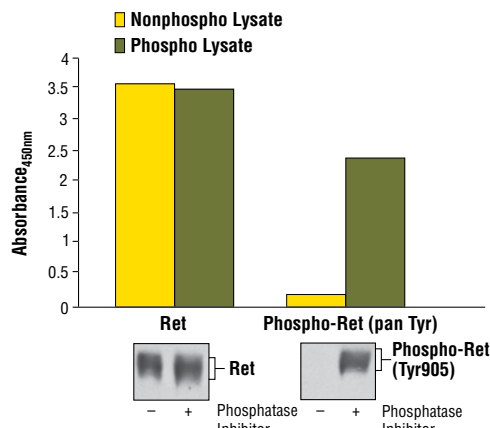


Figure 1: Constitutive phosphorylation of Ret in TT cells lysed in the presence of phosphatase inhibitors\* (phospho lysate) is detected by PathScan® Phospho-Ret (pan Tyr) Sandwich ELISA Kit #7034 (upper, right). In contrast, a low level of phospho-Ret protein is detected in TT cells lysed in the absence 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,  $\beta$ -glycerophosphate and  $\text{Na}_3\text{VO}_4$ .

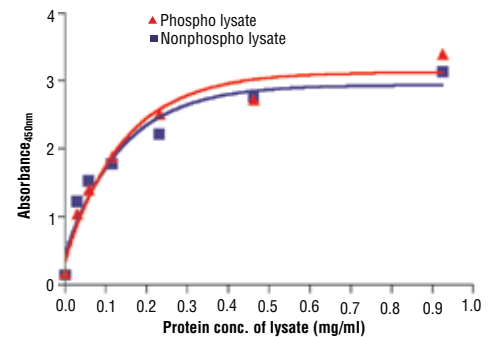


Figure 2: The relationship between protein concentration of phospho or nonphospho lysates and the absorbance at 450 nm is shown. TT cells were cultured (85% confluence) and lysed with or without the addition of phosphatase inhibitor to the lysis buffer (phospho or nonphospho lysate).

## 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<sub>3</sub>VO<sub>4</sub>, 1 μg/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 in diameter) 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. Generally, 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. However, dilution factors need to be titrated when specific cell lysates are used.

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 4.
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 4.
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.