

**#7968C** Store at 4°C

# PathScan® Phospho-HER2/ErbB2 (panTyr) Sandwich ELISA Kit



1 Kit  
 (96 assays)

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

## Species Cross-Reactivity: H

**Description:** The PathScan® Phospho-HER2/ErbB2 (panTyr) Sandwich ELISA Kit is a solid phase sandwich enzyme-linked immunosorbent assay (ELISA) that detects endogenous levels of tyrosine-phosphorylated HER2/ErbB2 protein. A HER2/ErbB2 rabbit mAb has been coated on the microwells. After incubation with cell lysates, HER2/ErbB2 protein (phospho and nonphospho) is captured by the coated antibody. Following extensive washing, a phosphotyrosine mouse detection antibody is added to detect captured tyrosine-phosphorylated HER2/ErbB2 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 HER2/ErbB2 protein phosphorylated on tyrosine.

Antibodies in kit are custom formulations specific to kit.

**Specificity/Sensitivity:** PathScan® Phospho-HER2/ErbB2 (panTyr) Sandwich ELISA Kit #7968 detects endogenous levels of HER2/ErbB2 protein only when phosphorylated at Tyr residues (see Figure 1). The kit sensitivity is shown in Figure 2. This kit does not cross-react with other proteins of ErbB family (data not shown). This kit detects proteins from the indicated species, as determined through in-house testing, but may also detect homologous proteins from other species.

**Background:** The ErbB2 (HER2) proto-oncogene encodes a 185 kDa transmembrane, receptor-like glycoprotein with intrinsic tyrosine kinase activity (1). While ErbB2 lacks an identified ligand, ErbB2 kinase activity can be activated in the absence of a ligand when overexpressed and through heteromeric associations with other ErbB family members (2). Amplification of the ErbB2 gene and overexpression of its product are detected in almost 40% of human breast cancers (3). Binding of the c-Cbl ubiquitin ligase to ErbB2 at Tyr1112 leads to ErbB2 poly-ubiquitination and enhances degradation of this kinase (4). ErbB2 is a key therapeutic target in the treatment of breast cancer and other carcinomas and targeting the regulation of ErbB2 degradation by the c-Cbl-regulated proteolytic pathway is one potential therapeutic strategy. Phosphorylation of the kinase domain residue Tyr877 of ErbB2 (homologous to Tyr416 of pp60c-Src) may be involved in regulating ErbB2 biological activity. The major autophosphorylation sites in ErbB2 are Tyr1248 and Tyr1221/1222; phosphorylation of these sites couples ErbB2 to the Ras-Raf-MAP kinase signal transduction pathway (1,5).

Product Includes	Item #	Kit Quantity	Color	Storage Temp
HER2/ErbB2 Rabbit mAb Coated Microwells*	47017	96 tests		4°C
Phospho-Tyrosine Mouse Detection mAb	12982	1 each	Green (Lyophilized)	4°C
Anti-mouse IgG, HRP-linked Antibody (ELISA Formulated)	13304	1 each	Red (Lyophilized)	4°C
Detection Antibody Diluent	13339	11 ml	Green	4°C
HRP Diluent	13515	11 ml	Red	4°C
TMB Substrate	7004	11 ml		4°C
STOP Solution	7002	11 ml		4°C
Sealing Tape	54503	2 sheets		4°C
ELISA Wash Buffer (20X)	9801	25 ml		4°C
ELISA Sample Diluent	11083	25 ml	Blue	4°C
Cell Lysis Buffer (10X)	9803	15 ml		-20°C

\*12 8-well modules – Each module is designed to break apart for 8 tests.

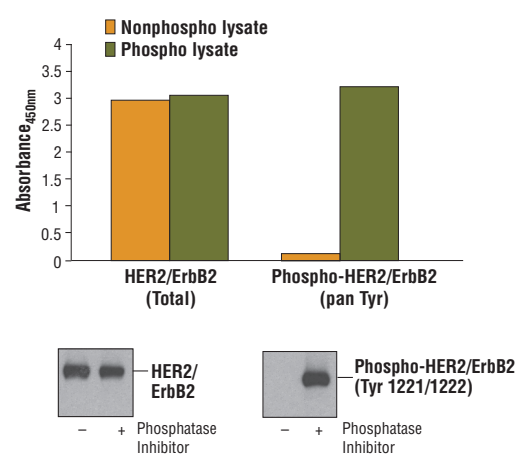


Figure 1: Constitutive phosphorylation of HER2/ErbB2 in Calu-3 cells lysed in the presence of phosphatase inhibitors\* (phospho lysate) is detected by PathScan® Phospho-HER2/ErbB2 (panTyr) Sandwich ELISA Kit #7968 (top, right). In contrast, a low level of phospho-HER2/ErbB2 protein is detected in Calu-3 cells lysed in the absence of phosphatase inhibitors\* (nonphospho lysate). Similar levels of total HER2/ErbB2 protein from both nonphospho or phospho lysates are detected by PathScan® Total HER2/ErbB2 Sandwich ELISA Kit #7310 (top, left). Absorbance at 450 nm is shown in the top figure while corresponding western blots using Phospho-HER2/ErbB2 (Tyr1221/1222) Rabbit mAb #2243 (right) or a total HER2/ErbB2 Antibody #2242 (left) are shown in the bottom figure.

\*Phosphatase inhibitors include sodium pyrophosphate, β-glycerophosphate and Na<sub>3</sub>VO<sub>4</sub>.

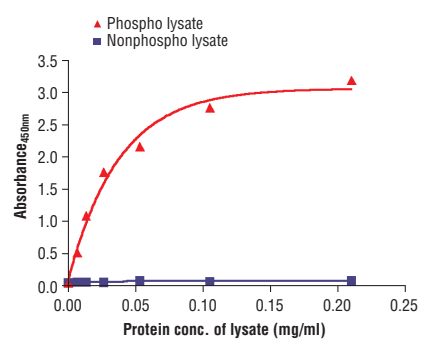


Figure 2: The relationship between protein concentration of phospho or nonphospho lysates and the absorbance at 450 nm is shown. Unstarved Calu-3 cells were cultured (85% confluence) and lysed with or without the addition of phosphatase inhibitor to the lysis buffer (phospho or nonphospho lysate).

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**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 All—all species expected Species enclosed in parentheses are predicted to react based on 100% sequence homology.

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

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

### A Solutions and Reagents

**NOTE:** Prepare solutions with purified water.

- Microwell strips:** Bring all to room temperature before use.
- 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.
- 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.
- Detection Antibody Diluent:** Green colored diluent for reconstitution and dilution of the detection antibody (11 ml provided).
- HRP Diluent:** Red colored diluent for reconstitution and dilution of the HRP-Linked Antibody (11 ml provided).
- Sample Diluent:** Blue colored diluent provided for dilution of cell lysates.
- 1X Wash Buffer:** Prepare by diluting 20X Wash Buffer (included in each PathScan® Sandwich ELISA Kit) in purified water.
- 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 phenylmethyl-sulfonyl fluoride (PMSF) immediately before use.
- TMB Substrate** (#7004).
- 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.**

- Aspirate media when the culture reaches 80–90% confluence. Treat cells by adding fresh media containing regulator for desired time.
- Remove media and rinse cells once with ice-cold 1X PBS.
- 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.
- Scrape cells off the plate and transfer to an appropriate tube. Keep on ice.
- Sonicate lysates on ice.
- 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**

- Remove media by low speed centrifugation (~1200 rpm) when the culture reaches 0.5–1.0 × 10<sup>6</sup> viable cells/ml. Treat cells by adding fresh media containing regulator for desired time.
- Collect cells by low speed centrifugation (~1200 rpm) and wash once with 5–10 ml ice-cold 1X PBS.
- Cells harvested from 50 ml of growth media can be lysed in 2.0 ml of 1X Cell Lysis Buffer plus 1 mM PMSF.
- Sonicate lysates on ice.
- 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

- 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.
- 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.
- 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.
- Gently remove the tape and wash wells:
  - Discard plate contents into a receptacle.
  - Wash 4 times with 1X Wash Buffer, 200 µl each time for each well.
  - 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.
  - Clean the underside of all wells with a lint-free tissue.
- 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.
- Repeat wash procedure (Section C, Step 4).
- 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.
- Repeat wash procedure (Section C, Step 4).
- 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.
- 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.

- Read results.
  - Visual Determination:** Read within 30 min after adding STOP Solution.
  - Spectrophotometric Determination:** Wipe underside of wells with a lint-free tissue. Read absorbance at 450 nm within 30 min after adding STOP Solution.

#### Background References:

- (1) Muthuswamy, S.K. et al. (1999) *Mol. Cell. Biol.* 19, 6845–6857.
- (2) Qian, X. et al. (1994) *Proc. Natl. Acad. Sci. USA* 91, 1500–1504.
- (3) Dittadi, R. and Gion, M. (2000) *J. Natl. Cancer Inst.* 92, 1443–1444.
- (4) Klapper, L.N. et al. (2000) *Cancer Res.* 60, 3384–3388.
- (5) Kwon, Y.K. et al. (1997) *J. Neurosci.* 17, 8293–8299.