

# PathScan® Phospho-HER3/ErbB3 (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-HER3/ErbB3 (panTyr) Sandwich ELISA Kit is a solid phase sandwich enzyme-linked immunosorbent assay (ELISA) that detects endogenous levels of tyrosine-phosphorylated HER3/ErbB3 protein. A HER3/ErbB3 rabbit mAb has been coated on the microwells. After incubation with cell lysates, HER3/ErbB3 protein (phospho and nonphospho) is captured by the coated antibody. Following extensive washing, a phospho-tyrosine mouse detection antibody is added to detect captured tyrosine-phosphorylated HER3/ErbB3 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 HER3/ErbB3 protein phosphorylated on tyrosine.

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

**Specificity/Sensitivity:** PathScan® Phospho-HER3/ErbB3 (panTyr) Sandwich ELISA Kit #7890 detects endogenous levels of HER3/ErbB3 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:** HER3/ErbB3 is a member of the ErbB receptor protein tyrosine kinase family, but lacks tyrosine kinase activity. Tyrosine phosphorylation of ErbB3 depends on its association with other ErbB tyrosine kinases. Upon ligand binding, heterodimers form between ErbB3 and other ErbB proteins and ErbB3 is phosphorylated on tyrosine residues by the activated ErbB kinase (1,2). There are at least 9 potential tyrosine phosphorylation sites in the carboxy-terminal tail of ErbB3. These sites serve as consensus binding sites for signal transducing proteins, including Src family members, Grb2, and the p85 subunit of PI3 kinase, which mediate ErbB downstream signaling (3). Both Tyr1222 and Tyr1289 of ErbB3 reside within a YXXM motif and participate in signaling to PI3 kinase (4).

ErbB3 is highly expressed in many cancer cells (5) and activation of the ErbB3/PI3K pathway is correlated with malignant phenotypes of adenocarcinomas (6). In tumor development, ErbB3 may function as an oncogenic unit together with other ErbB members (e.g. ErbB2 requires ErbB3 to drive breast tumor cell proliferation) (7). Thus, inhibiting interaction between ErbB3 and ErbB tyrosine kinases has become a novel strategy for anti-tumor therapy.

Product Includes	Item #	Kit Quantity	Color	Storage Temp
HER3/ErbB3 Rabbit mAb Coated Microwells*	97125	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.

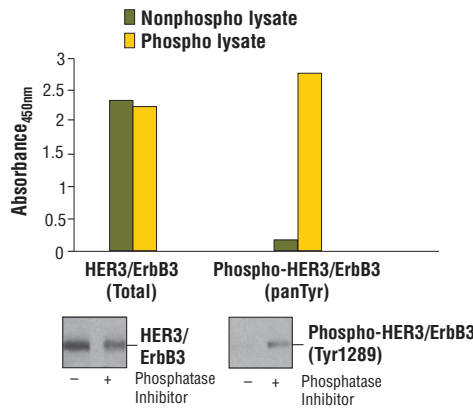


Figure 1: Constitutive phosphorylation of HER3/ErbB3 in Calu-3 cells lysed in the presence of phosphatase inhibitors\* (phospho lysate) is detected by PathScan® Phospho-HER3/ErbB3 (panTyr) Sandwich ELISA Kit #7890 (top, right). In contrast, a low level of phospho-HER3/ErbB3 protein is detected in Calu-3 cells lysed in the absence of phosphatase inhibitors\* (nonphospho lysate). Similar levels of total HER3/ErbB3 protein from both nonphospho or phospho lysates are detected by PathScan® Total HER3/ErbB3 Sandwich ELISA Kit #7888 (top, left). Absorbance at 450 nm is shown in the top figure while corresponding western blots using Phospho-HER3/ErbB3 (Tyr1289) Rabbit mAb #4791 (right) or a total HER3/ErbB3 Antibody #4754 (left) are shown in the bottom figure.

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

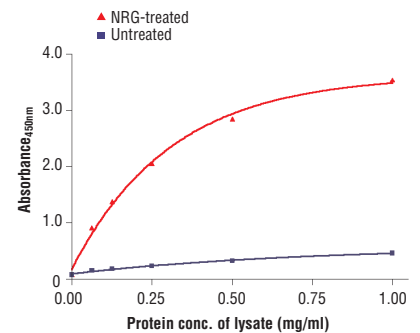


Figure 2: The relationship between protein concentration of lysates from untreated and NRG-treated T-47D cells and kit assay optical density readings. After starvation, T-47D cells (85% confluence) were treated with Human Neuregulin-1 (hNRG-1) #5218 (100 ng/ml) for 5 minutes at 37°C and then lysed.

## 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 or 1X Cell Lysis Buffer #7018: 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.
- 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) Yarden, Y. and Slivkowsky, M.X. (2001) *Nature Rev. Mol. Cell. Biol.* 2, 127-137.
- (2) Guy, P.M. et al. (1994) *Proc. Natl. Acad. Sci. USA* 91, 8132-8136.
- (3) Songyang, Z. et al. (1993) *Cell* 72, 767-778.
- (4) Kim, H.H. et al. (1994) *J. Biol. Chem.* 269, 24747-24755.
- (5) Sithanandam, G. et al. (2003) *Carcinogenesis* 24, 1581-1592.
- (6) Kobayashi, M. et al. (2003) *Oncogene* 22, 1294-1301.
- (7) Holbro, T. et al. (2003) *Proc. Natl. Acad. Sci. USA* 100, 8933-8938.