

PathScan® Total HER3/ErbB3 Sandwich ELISA Kit

✓ 1 Kit
(96 assays)

Orders ■ 877-616-CELL (2355)
orders@cellsignal.com

Support ■ 877-678-TECH (8324)
info@cellsignal.com

Web ■ www.cellsignal.com

New 06/09

This product is for *in vitro* research use only and is not intended for use in humans or animals.
This product is not intended for use as a therapeutic or in diagnostic procedures.

Entrez-Gene ID #2065
Swiss-Prot Acc. # P21860

Species Cross-Reactivity: H

Description: The PathScan® Total HER3/ErbB3 Sandwich ELISA Kit is a solid phase sandwich enzyme-linked immunosorbent assay (ELISA) that detects endogenous levels of HER3/ErbB3 protein. A HER3/ErbB3 mouse antibody 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 HER3/ErbB3 rabbit antibody is added to detect captured HER3/ErbB3 protein. Anti-rabbit 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.

Specificity/Sensitivity: PathScan® Total HER3/ErbB3 Sandwich ELISA Kit #7888 detects endogenous levels of total HER3/ErbB3 protein in human cells, as shown in 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).

Please visit www.cellsignal.com for a complete listing of recommended companion products.

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/PI3 kinase 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, prevention of the interaction between ErbB3 and ErbB tyrosine kinases has become a novel anti-tumor strategy.

Products Included	Volume	Solution Color
HER3/ErbB3 Mouse Antibody Coated Microwells*	96 tests	
HER3/ErbB3 Rabbit Detection Antibody	11 ml	green
Anti-rabbit 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).

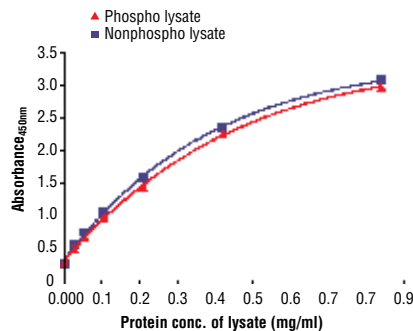


Figure 2: The relationship between protein concentration of phospho or nonphospho lysates and the absorbance at 450 nm is shown. Calu-3 cells were cultured (85% confluence) and lysed with or without the addition of phosphatase inhibitor to the lysis buffer (phospho or nonphospho lysate).

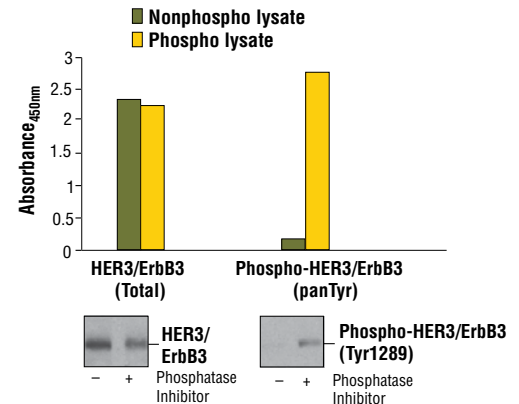


Figure 1: Constitutive phosphorylation of HER3/ErbB3 in Calu-3 cells lysed in the presence of phosphatase inhibitors* (phospho lysate) is detected by PathScan® Phospho-HER3/ErbB3 (panTyr) Sandwich ELISA Kit #7890 (top, right). In contrast, a low level of phospho-HER3/ErbB3 protein is detected in Calu-3 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) HER3/ErbB3 Antibody #4754 (left) are shown in the bottom figure.
* Phosphatase inhibitors include sodium pyrophosphate, β -glycerophosphate and Na_3VO_4 .

Background References:

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

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.

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 Na₃VO₄, 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.