

PathScan® Phospho-HER2/ErbB2 (Tyr1221/1222) Sandwich ELISA Antibody Pair

✓ 1 Kit
(4 X 96 assays)



Orders ■ 877-616-CELL (2355)
orders@cellsignal.com
Support ■ 877-678-TECH (8324)
info@cellsignal.com
Web ■ www.cellsignal.com

New 11/08

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 #2064
Swiss-Prot Acc. #P04626

Species Cross-Reactivity: H

Description: CST's PathScan® Phospho-HER2/ErbB2 (Tyr1221/1222) Sandwich ELISA Antibody Pair is being offered as an economical alternative to our PathScan® Phospho-HER2/ErbB2 (Tyr1221/1222) Sandwich ELISA Kit #7148. Capture and Detection antibodies (100X stocks) and HRP-conjugated secondary antibody (1000X stock) are supplied. Sufficient reagents are supplied for 4 x 96 well ELISAs. The HER2/ErbB2 Capture Antibody is coated on a 96 well microplate in PBS overnight. After blocking, cell lysates are added followed by a Phospho-HER2/ErbB2 (Tyr1221/1222) Detection Antibody and anti-rabbit IgG, HRP conjugated antibody. HRP substrate, TMB, is added for color development. The magnitude of the absorbance for this developed color is proportional to the quantity of Phospho-HER2/ErbB2 (Tyr1221/1222) protein.

Reagents not supplied:

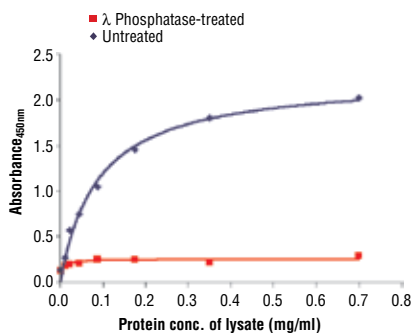
- Phosphate Buffered Saline (PBS-20X) #9808
- Phosphate Buffered Saline with Tween-20 (PBST-20X) #9809
- Cell Lysis Buffer (10X) #9803
- TMB Substrate #7004
- STOP Solution #7002
- Blocking Buffer: 1X PBS/0.05% Tween-20, 1% BSA
- 96 Well Microplates**
- Microplate Reader

** Antibody Pairs have been validated on Corning® 96 Well Clear Polystyrene High Bind Stripwell™ Microplates (#2592) and Corning® 96 Well EIA/RIA Easy Wash™ Clear Flat Bottom Polystyrene High Bind Microplates (#3369).

Notes: Antibody pairs have been optimized using recommended buffers, reagents, plates and the included protocol. Solutions should be made fresh daily.

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

Products Included	Volume	Cap Color	Storage
HER2/ErbB2 Mouse Capture Antibody (100X)	0.4 ml	Pink	4°C
HER2/ErbB2 (Tyr1221/1222) Rabbit Detection Antibody (100X)	0.4 ml	Blue	4°C
Anti-rabbit IgG, HRP-Linked Antibody (1000X)	0.04 ml	Red	-20°C



The relationship between protein concentration of untreated and λ phosphatase-treated Calu-3 cell lysates, and the absorbance at 450 nM using PathScan® Phospho-HER2/ErbB2 (Tyr1221/1222) Sandwich ELISA Antibody Pair #7817 is shown.

with the regulation of ErbB2 degradation by the c-Cbl-regulated proteolytic pathway as 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).

Storage: Capture and detection antibodies are stored at 4°C. Anti-rabbit IgG, HRP-linked antibody is stored at -20°C.

Companion Products:

- Phosphate Buffered Saline (PBS-20X) #9808
- Phosphate Buffered Saline with Tween 20 (PBST-20X) #9809
- BSA #9998
- TMB Substrate #7004
- STOP Solution #7002
- Cell Lysis Buffer (10X) #9803
- Anti-rabbit IgG, HRP-linked Antibody #7074
- PathScan® Phospho-HER2/ErbB2 (Tyr1221/1222) Sandwich ELISA Kit #7148
- HER2/ErbB2 (29D8) Rabbit mAb #2165
- HER2/ErbB2 (44E7) Mouse mAb #2248
- HER2/ErbB2 (M45) Antibody #3250
- HER2/ErbB2 Antibody #2242
- Phospho-HER2/ErbB2 (Tyr1221/1222) (6B12) Rabbit mAb #2243
- Phospho-HER2/ErbB2 (Tyr1221/1222) Antibody #2249

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.

PathScan® Sandwich ELISA Antibody Pair Protocol

A Required Reagents

- Coating Buffer:** 1X PBS, (20X PBS #9808)
3.2 mM Na₂HPO₄, 0.5 mM KH₂PO₄, 1.3 mM KCl, 135 mM NaCl, pH 7.4
- Wash Buffer:** 1X PBS/0.05% Tween-20, (20X PBST #9809)
- Blocking Buffer:** 1X PBS/0.05% Tween-20, 1% BSA
- 1X Cell Lysis Buffer:** (10X Cell Lysis Buffer #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.
- TMB Substrate:** (TMB Substrate #7004)
- STOP Solution:** (STOP Solution #7002)

NOTE: Reagents should be made fresh daily

B Coating Procedure

- Rinse microplate with dH₂O. Add 200 μl of dH₂O and discard liquid. Blot on paper towel to make sure wells are dry.
- Dilute capture antibody 1:100 in PBS. For a single 96 well plate, add 100 μl of Capture Antibody Stock to 9.9 ml PBS. Mix well and add 100 μl/well. Cover plate and incubate overnight at 4°C (17-20 hours).
- After overnight coating, gently uncover plate and wash wells:**
 - Discard plate contents into a receptacle.
 - Wash 4 times with 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.
- Block plates. Add 150 μl of Blocking Buffer/well, cover plate and incubate at 37°C for 2 hours.
- After blocking, wash plate as in Step 3. Plate is ready to use.

C Preparing Cell Lysates

- Aspirate media, treat cells by adding fresh media containing regulator for desired time.
- To harvest cells under nondenaturing conditions, remove media and rinse cells once with ice-cold PBS.
- Remove PBS and add 0.5 ml ice-cold 1X Cell Lysis Buffer plus 1 mM phenylmethylsulfonyl fluoride (PMSF) to each plate (10 cm diameter plate) and incubate the plate on ice for 5 minutes.
- Scrape cells off the plate and transfer to an appropriate tube. Keep on ice.
- Sonicate lysates on ice.
- 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.

D Test Procedure

- Lysates can be used undiluted or diluted in Blocking Buffer. 100 μl of lysate is added per well. Cover plate and incubate at 37°C for 2 hours.
- Wash plate as in Coating Procedure, Step 3.
- Dilute detection antibody 1:100 in Blocking Buffer. For a single 96 well plate, add 100 μl of Detector Antibody Stock to 9.9 ml of Blocking Buffer. Mix well and add 100 μl/well. Cover plate and incubate at 37°C for 1 hour.
- Plate is washed as in Coating Procedure, Step 3.
- Secondary antibody, either, anti-mouse or anti-rabbit-HRP, is diluted 1:1000 in Blocking Buffer. For a single 96 well plate, add 10 μl of secondary antibody stock to 9.99 ml of Blocking Buffer. Mix well and add 100 μl/well. Cover and incubate at 37°C for 30 minutes.
- Wash plate as in Coating Procedure, Step 3.
- Add 100 μl of TMB Substrate per well. Cover and incubate at 37°C for 10 minutes.
- Add 100 μl of STOP Solution per well.
- Read plate on a microplate reader at Absorbance 450 nm.