

PathScan® Phospho-EGF Receptor (Tyr845) 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

rev. 05/19/11

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.

Species Cross-Reactivity: H

Introduction: CST's PathScan® Phospho-EGF Receptor (Tyr845) Sandwich ELISA Kit is a solid phase sandwich enzyme-linked immunosorbent assay (ELISA) that detects endogenous levels of phospho-EGF Receptor (Tyr845) protein. A EGF Receptor Mouse mAb has been coated onto the microwells. After incubation with cell lysates, both phospho- and nonphospho-EGF Receptor proteins are captured by the coated antibody. Following extensive washing, Phospho-EGF Receptor (Tyr845) Rabbit mAb is added to detect the captured phospho-EGF Receptor protein. Anti-rabbit IgG, HRP-linked Antibody #7074* is then used to recognize the bound detection antibody. HRP substrate, TMB, is added to develop color. The magnitude of optical density for this developed color is proportional to the quantity of phospho-EGF Receptor (Tyr845) protein.

* Antibodies in kit are custom formulations specific to kit.

Specificity/Sensitivity: CST's PathScan® Phospho-EGF Receptor (Tyr845) Sandwich ELISA Kit detects endogenous levels of phospho-EGF Receptor (Tyr845) protein. As shown in figure 1, using the Phospho-EGF Receptor (Tyr845) ELISA Kit #7189, a significant induction of Phospho-EGF Receptor (Tyr845) in A-431 cells treated with EGF is detected. However, the levels of EGF Receptor (phospho and non-phospho) detected by PathScan® Total EGF Receptor Sandwich ELISA Kit #7250, remain unchanged.

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

Products Included	Volume	Solution Color
EGF Receptor Mouse mAb Coated Microwells*	96 tests	
Phospho-EGF Receptor (Tyr845) Detection Ab	11 ml	green
Anti-rabbit IgG HRP-Linked Ab	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).

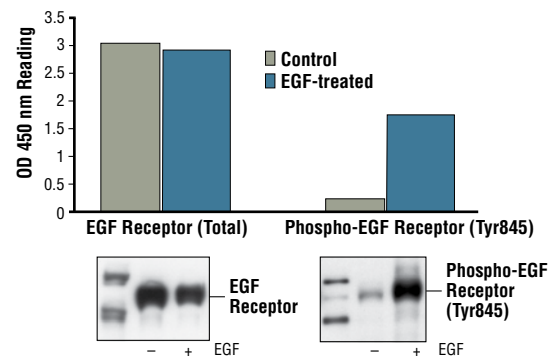


Figure 1: Treatment of A431 cells with EGF stimulates phosphorylation of EGF Receptor at Tyr845, detected by PathScan® Phospho-EGF Receptor (Tyr845) Sandwich ELISA kit #7189, but does not affect the level of total EGF Receptor detected by PathScan® Total EGF Receptor Sandwich ELISA kit #7250. OD 450 nm readings are shown in the top figure, while the corresponding Western blot using Phospho-EGF Receptor (Tyr845) Antibody #2231 (right panel) or EGF Receptor Antibody #2232 (left panel), is shown in the bottom figure.



Background: Epidermal growth factor (EGF) receptor is a 170 kDa tyrosine kinase. Ligand binding results in receptor dimerization, autophosphorylation, activation of downstream signaling and lysosomal degradation (1,2). Phosphorylation of Tyr845 in the kinase domain is implicated in stabilizing the activation loop, maintaining the enzyme in an active state and providing a binding surface for substrate proteins (3,4). c-Src is involved in phosphorylation of Tyr845 (5). Phospho-tyrosine 992 is a direct binding site for the PLC γ SH2 domain, resulting in activation of PLC γ -mediated downstream signaling (6). Phosphorylation of Tyr1045 creates a major docking site for c-Cbl (7). Binding of c-Cbl to the activated EGFR leads to receptor ubiquitination and degradation (8). Phospho-Tyr1068 of activated EGFR is a direct binding site for Grb2 (9). Phospho-tyrosine 1148 and 1173 provide a docking site for SHC (2). Both sites are involved in the activation of MAP kinase signaling. Phosphorylation of EGFR on serine and threonine residues attenuates EGFR kinase activity. Serines 1046 and 1047 in the carboxy-terminal region of EGFR are sites phosphorylated by CaM kinase II. Mutations to either serine 1046 or 1047 upregulate tyrosine autokinase activity of EGFR (10).

Background References:

- (1) Hackel, P.O. et al. (1999) *Curr. Opin. Cell Biol.* 11, 184–189.
- (2) Zwick, E. et al. (1999) *Trends Pharmacol. Sci.* 20, 408–412.
- (3) Cooper, J.A. and Howell, B. (1993) *Cell* 73, 1051–1054.
- (4) Hubbard, S.R. et al. (1994) *Nature* 372, 746–754.
- (5) Biscardi, J.S. et al. (1999) *J. Biol. Chem.* 274, 8335–8343.
- (6) Emlét, D.R. et al. (1997) *J. Biol. Chem.* 272, 4079–4086.
- (7) Levkowitz, G. et al. (1999) *Mol. Cell* 4, 1029–1040.
- (8) Ettenberg, S.A. et al. (1999) *Oncogene* 18, 1855–1866.
- (9) Rojas, M. et al. (1996) *J. Biol. Chem.* 271, 27456–27461.
- (10) Feinmesser, R.L. et al. (1999) *J. Biol. Chem.* 274, 16168–16173.

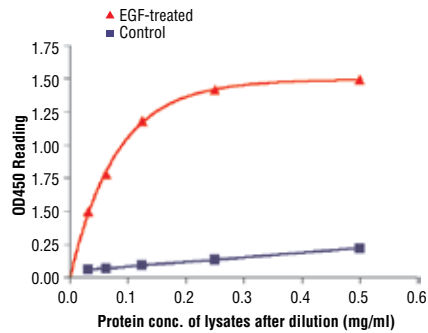


Figure 2: The relationship between protein concentration of lysates from untreated and EGF-treated A431 cells and kit assay optical density readings. After starvation, A431 cells (85% confluence) were treated with EGF (100 ng/ml) for 5 min at 37°C, and then lysed.

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_3VO_4 , 1 $\mu\text{g}/\text{ml}$ 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 cm²) 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. (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.

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 C4.
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 C4.
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.