

PathScan® Total EGF Receptor Chemiluminescent Sandwich ELISA Kit



Cell Signaling
TECHNOLOGY®

- ✓ 1 Kit
(96 assays)
Low volume microplate

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New 12/11

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.

Entrez-Gene ID #1956
Swiss-Prot Acc. #P00533

Species Cross-Reactivity: H

Description: PathScan® Total EGF Receptor Chemiluminescent Sandwich ELISA Kit is a solid phase sandwich enzyme-linked immunosorbent assay (ELISA) that detects endogenous levels of total EGF receptor protein. Chemiluminescent ELISAs often have a wider dynamic range and higher sensitivity than conventional chromogenic detection. This chemiluminescent ELISA, which is offered in low volume microplates, shows increased signal and sensitivity while using a smaller sample size. An 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, EGF Receptor Rabbit mAb is added to detect both the captured phospho- and nonphospho-EGF receptor protein. Anti-rabbit IgG, HRP-linked Antibody #7074 is then used to recognize the bound detection antibody. Chemiluminescent reagent is added for signal development. The magnitude of light emission, measured in relative light units (RLU), is proportional to the quantity of total EGF receptor protein.

Antibodies in kit are custom formulations specific to kit.

Specificity/Sensitivity: PathScan® Total EGF Receptor Chemiluminescent Sandwich ELISA Kit #7297 detects endogenous levels of EGF receptor protein. 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 epidermal growth factor (EGF) receptor is a transmembrane tyrosine kinase that belongs to the HER/ErbB protein family. Ligand binding results in receptor dimerization, autophosphorylation, activation of downstream signaling, internalization, and lysosomal degradation (1,2). Phosphorylation of EGF receptor (EGFR) at Tyr845 in the kinase domain is implicated in stabilizing the activation loop, maintaining the active state enzyme, and providing a binding surface for substrate proteins (3,4). c-Src is involved in phosphorylation of EGFR at Tyr845 (5). The SH2 domain of PLC-γ binds at phospho-Tyr992, resulting in activation of PLC-γ-mediated downstream signaling (6). Phosphorylation of EGFR at Tyr1045 creates a major docking site for the adaptor protein c-Cbl, leading to receptor ubiquitination and degradation following EGFR activation (7,8). The GRB2 adaptor protein binds activated EGFR at phospho-Tyr1068 (9). A pair of phosphorylated EGFR residues (Tyr1148 and Tyr1173) provide a docking site for the Shc scaffold protein, with both sites involved in MAP kinase signaling activation (2). Phosphorylation of EGFR at specific serine and threonine residues attenuates EGFR kinase activity. EGFR carboxy-terminal residues Ser1046 and Ser1047 are phosphorylated by CaM kinase II; mutation of either of these serines results in upregulated EGFR tyrosine autophosphorylation (10).

Products Included	Volume	Solution Color
EGF Receptor Mouse mAb Coated Microwells*	96 tests	
EGF Receptor Rabbit Detection mAb	5.5 ml	Green
Anti-rabbit IgG, HRP-linked Antibody	5.5 ml	Red
Luminol/Enhancer Solution	3 ml	Colorless
Stable Peroxide Buffer	3 ml	Colorless
Sealing Tape	2 sheets	
20X Wash Buffer	25 ml	Colorless
Sample Diluent	25 ml	Blue
**Cell Lysis Buffer #9803	15 ml	Yellowish

Low volume microplate * 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).

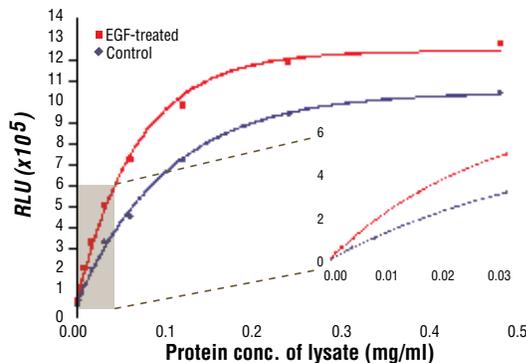


Figure 1. The relationship between protein concentration of lysates from A-431 cells, untreated or treated with hEGF #8916, and immediate light generation with chemiluminescent substrate. After starvation, A-431 cells (85% confluence) were treated with hEGF (100 ng/ml, 5 min at 37°C) and then lysed. Graph inset corresponding to the shaded area shows high sensitivity and a linear response at the low protein concentration range.

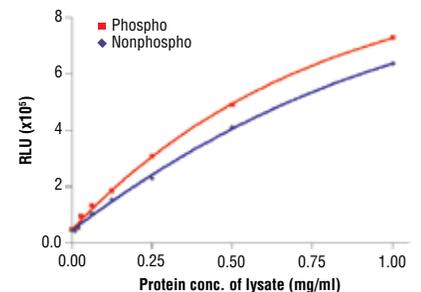


Figure 2. The relationship between protein concentration of lysates prepared using H1975 cells, lysed with (phospho) and without (nonphospho) the addition of phosphatase inhibitors to the lysis buffer, and immediate light generation using chemiluminescent substrate.

Background References:

- (1) Hackel, P.O. et al. (1999) *Curr Opin Cell Biol* 11, 184-9.
- (2) Zwick, E. et al. (1999) *Trends Pharmacol Sci* 20, 408-12.
- (3) Cooper, J.A. and Howell, B. (1993) *Cell* 73, 1051-4.
- (4) Hubbard, S.R. et al. (1994) *Nature* 372, 746-54.
- (5) Biscardi, J.S. et al. (1999) *J Biol Chem* 274, 8335-43.
- (6) Emlet, D.R. et al. (1997) *J Biol Chem* 272, 4079-86.
- (7) Levkowitz, G. et al. (1999) *Mol Cell* 4, 1029-40.
- (8) Ettenberg, S.A. et al. (1999) *Oncogene* 18, 1855-66.
- (9) Rojas, M. et al. (1996) *J Biol Chem* 271, 27456-61.
- (10) Feinmesser, R.L. et al. (1999) *J Biol Chem* 274, 16168-73.

Chemiluminescent ELISA Protocol

NOTE: This chemiluminescent ELISA is offered in low volume microplate. Samples and reagents only require 50 μ l per microwell.

A Required Reagents

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: (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 Na_3VO_4 , 1 μ g/ml leupeptin. This buffer can be stored at 4°C for short-term use (1–2 weeks).
Note: CST recommends adding 1 mM PMSF immediately before use.

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 50 μ l of Sample Diluent (supplied in each Pathscan® Sandwich ELISA Kit, blue color) to a microcentrifuge tube. Transfer 50 μ l 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 50 μ l 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 room temperature (RT). 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, 150 μ l 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 dry completely at any time.
 - d. Clean the underside of all wells with a lint-free tissue.
5. Add 50 μ l of Detection Antibody (green color) to each well. Seal with tape and incubate the plate for 1 hour at room temperature.
6. Repeat wash procedure as in Step C4.
7. Add 50 μ l of HRP-linked secondary antibody (red color) to each well. Seal with tape and incubate the plate for 30 minutes at room temperature.
8. Repeat wash procedure as in Step C4.
9. Prepare Working Solution by mixing equal parts Luminol/Enhancer Solution and Stable Peroxide Buffer.
10. Add 50 μ l of the Working Solution to each well.

Use a plate-based luminometer to measure Relative Light Units (RLU) at 425nM within 1-10 minutes following addition of the substrate.

Optimal signal intensity is achieved when read within 10 minutes.