

#7036C Store at 4°C

# PathScan® Phospho-EGF Receptor (Tyr1068) Chemiluminescent Sandwich ELISA Kit



1 Kit  
 (96 assays)  
 Low volume microplate

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rev. 05/24/19

**For Research Use Only. Not For Use In Diagnostic Procedures.**

## Species Cross-Reactivity: H

**Description:** CST's PathScan® Phospho-EGF Receptor (Tyr1068) Chemiluminescent Sandwich ELISA Kit is a solid phase sandwich enzyme-linked immunosorbent assay (ELISA) that detects endogenous levels of phospho-EGF Receptor (Tyr1068) protein with a chemiluminescent read-out. 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, EGF receptor proteins (phospho and nonphospho) are captured by the coated antibody. Following extensive washing, Phospho-EGF Receptor (Tyr1068) 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. Chemiluminescent reagent is added for signal development. The magnitude of light emission, measured in relative light units (RLU), is proportional to the quantity of Phospho-EGF Receptor (Tyr1068).

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

**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 PLCγ binds at phospho-Tyr992, resulting in activation of PLCγ-mediated downstream signaling (6). Phosphorylation of EGFR at Tyr1045 creates a major docking site for c-Cbl, an adaptor protein that leads 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) provides 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).

**Specificity/Sensitivity:** PathScan® Phospho-EGF Receptor (Tyr1068) Chemiluminescent Sandwich ELISA Kit

| Product Includes  | Item # | Kit Quantity | Color               | Storage Temp |
|---|--------|--------------|---------------------|--------------|
| EGF Receptor MmAb Coated Microwells*                    | 99592  | 96 tests     |                     | 4°C          |
| P-EGF Receptor (Y1068) Rabbit Detection mAb             | 13019  | 1 each       | Green (Lyophilized) | 4°C          |
| Anti-rabbit IgG, HRP-linked Antibody (ELISA Formulated) | 13272  | 1 each       | Red (Lyophilized)   | 4°C          |
| Detection Antibody Diluent                              | 13339  | 5.5 ml       | Green               | 4°C          |
| HRP Diluent   | 13515  | 5.5 ml       | Red                 | 4°C          |
| Luminol/Enhancer Solution                               | 84850  | 3 ml         |                     | RT           |
| Stable Peroxide Buffer                                  | 42552  | 3 ml         |                     | RT           |
| 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        |

**Low volume microplate** \*12 8-well modules – Each module is designed to break apart for 8 tests.  
 Note: This kit contains components with mixed storage temperatures. Please store this entire kit at 4°C for long term storage. Upon first use, please store each component as indicated in the chart above and on individual component labels.

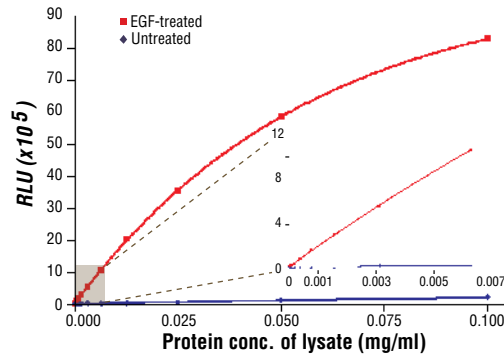


Figure 1. The relationship between protein concentration of lysates from A-431 cells, untreated or treated with hEGF #8916, and immediate light generation with chemiluminescent substrate. After starvation, A-431 cells (85% confluence) were treated with EGF (100 ng/ml, 5 min at 37°C) and then lysed. Graph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

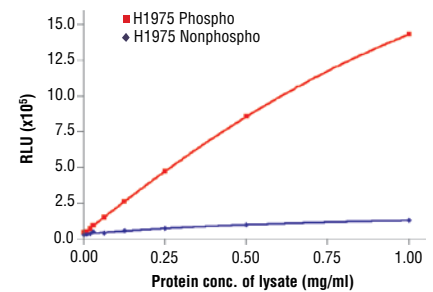


Figure 2. The relationship between protein concentration of lysates prepared using H1975 cells, lysed with (phospho) and without (nonphospho) the addition of phosphatase inhibitors to the lysis buffer, and immediate light generation using chemiluminescent substrate.

#7036 detects endogenous levels of phospho-EGF Receptor (Tyr1068) protein in human cells. This kit detects proteins from the indicated species, as determined through in-house testing, but may also detect homologous proteins from other species.

**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—zebra fish 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.

## PathScan® Chemiluminescent Sandwich ELISA Protocol (for kits with Lyophilized Antibodies)

**NOTE:** Refer to product-specific datasheets for assay incubation temperature. This chemiluminescent ELISA is offered in low volume microplates. Only 50 µl of samples or reagents are required in each microwell.

### 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 0.5 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 0.5 ml volume of reconstituted Detection Antibody to 5.0 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 0.5 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 0.5 ml volume of reconstituted HRP-Linked Antibody to 5.0 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 (5.5 ml provided).
- HRP Diluent:** Red colored diluent for reconstitution and dilution of the HRP-Linked Antibody (5.5 ml provided).
- Sample Diluent:** Blue colored diluent 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.
- Luminol/Enhancer Solution and Stable Peroxide Buffer**

\*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 x 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 50 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 room temperature. 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, 150 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 50 µl of reconstituted Detection Antibody (green color) to each well (refer to Section A, Step 2). Seal with tape and incubate the plate at room temperature for 1 hr.
- Repeat wash procedure (Section C, Step 4).
- Add 50 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 at room temperature for 30 min.
- Repeat wash procedure (Section C, Step 4).
- Prepare Detection Reagent Working Solution by mixing equal parts Luminol/Enhancer Solution and Stable Peroxide Buffer.
- Add 50 µl of the Detection Reagent Working Solution to each well.
- Use a plate-based luminometer to measure Relative Light Units (RLU) at 425 nm within 1–10 min following addition of the substrate. *Optimal signal intensity is achieved when read within 10 min.*

### Background References:

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