

PathScan® Total EGF Receptor Sandwich ELISA Kit



✓ 1 Kit
(96 Assays)

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

Species Cross-Reactivity: H

Description: CST's PathScan® Total EGF Receptor Sandwich ELISA Kit is a solid phase sandwich enzyme-linked immunosorbent assay (ELISA) that detects endogenous levels of total EGF Receptor protein. 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 Antibody is added to detect both the captured phospho- and nonphospho-EGF Receptor 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 optical density for this developed color is proportional to the quantity of total EGF Receptor protein.

Antibodies in kit are custom formulations specific to kit.

Specificity/Sensitivity: CST's PathScan® Total EGF Receptor Sandwich ELISA Kit #7250 detects endogenous levels of EGF Receptor Protein. As shown in Figure 1, using PathScan® Phospho-EGF Receptor (Tyr1068) ELISA Kit #7240, a significant induction of Phospho-EGF Receptor (Tyr1068) is detected in A-431 cells treated with EGF. The levels of total EGF Receptor (phospho and non-phospho) detected by PathScan® Total EGF Receptor Sandwich ELISA Kit #7250 remain unchanged. In Figure 3, western blot analysis of protein captured in the EGF Receptor mouse mAb coated microwell shows a single band corresponding to the EGF Receptor. 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 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 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

Product Includes	Item #	Kit Quantity	Color	Storage Temp
EGF Receptor Mouse mAb Coated Microwells*	10537	96 tests		4°C
EGF Receptor Rabbit Detection mAb	12951	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	11 ml	Green	4°C
HRP Diluent	13515	11 ml	Red	4°C
TMB Substrate	7004	11 ml		4°C
STOP Solution	7002	11 ml		4°C
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

*12 8-well modules – Each module is designed to break apart for 8 tests.

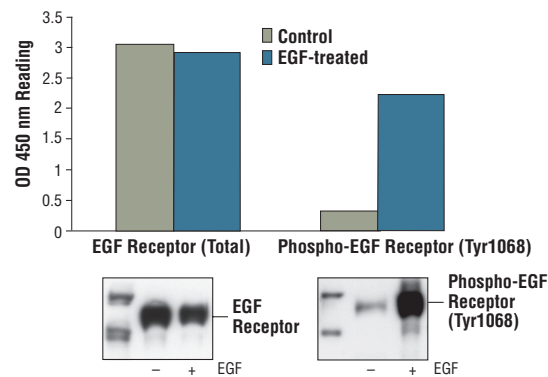


Figure 1: Treatment of A431 cells with EGF stimulates phosphorylation of EGF Receptor at Tyr1068, detected by PathScan® Phospho-EGF Receptor (Tyr1068) Sandwich ELISA kit #7240, but does not affect the level of total EGF Receptor detected by PathScan® Total EGF Receptor Sandwich ELISA kit #7250. OD 450nm readings are shown in the top figure, while the corresponding western blot using Phospho-EGF Receptor (Tyr1068) Antibody #2234 (right panel) or EGF Receptor Antibody #2232 (left panel), is shown in the bottom figure.

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).

U.S. Patent No. 5,675,063

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) Eittenberg, 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.

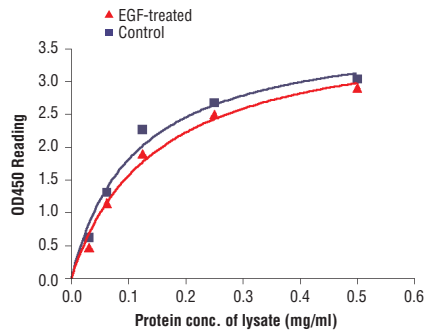


Figure 2: The relationship between protein concentration of lysates from untreated and EGF-treated A431 cells and kit assay optical density readings. After starvation, A431 cells (85% confluence) were treated with EGF (100 ng/ml) for 5 min at 37°C, and then lysed.

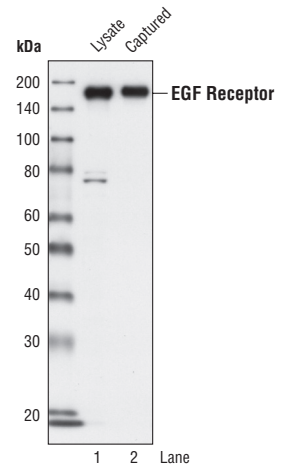


Figure 3: Kit specificity demonstrated by western blot analysis of the ELISA-well captured protein. Lysates prepared from human A431 cells were incubated in wells coated with an EGF Receptor capture antibody. Wells were washed and captured protein was solubilized in SDS gel loading buffer. A431 lysate (lane 1) and captured protein (lane 2) were analyzed by western blot using an EGF Receptor detection antibody. A single band corresponding to the EGF Receptor is detected in the captured material (lane 2).

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

A Solutions and Reagents

NOTE: Prepare solutions with purified water.

1. **Microwell strips:** Bring all to room temperature before use.
2. **Detection Antibody:** Supplied lyophilized as a green colored cake or powder. Add 1.0 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 1.0 ml volume of reconstituted Detection Antibody to 10.0 ml of Detection Antibody Diluent in a clean tube and gently mix. Unused working solution may be stored for 4 weeks at 4°C.
3. **HRP-Linked Antibody*:** Supplied lyophilized as a red colored cake or powder. Add 1.0 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 1.0 ml volume of reconstituted HRP-Linked Antibody to 10.0 ml of HRP Diluent in a clean tube and gently mix. Unused working solution may be stored for 4 weeks at 4°C.
4. **Detection Antibody Diluent:** Green colored diluent for reconstitution and dilution of the detection antibody (11 ml provided).
5. **HRP Diluent:** Red colored diluent for reconstitution and dilution of the HRP-Linked Antibody (11 ml provided).
6. **Sample Diluent:** Blue colored diluent provided for dilution of cell lysates.
7. **1X Wash Buffer:** Prepare by diluting 20X Wash Buffer (included in each PathScan® Sandwich ELISA Kit) in purified water.
8. **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.
9. **TMB Substrate** (#7004).
10. **STOP Solution** (#7002).

**Note: Some PathScan® ELISA Kits may include HRP-Linked Streptavidin in place of HRP-Linked Antibody.*

B Preparing Cell Lysates

For adherent cells.

1. Aspirate media when the culture reaches 80–90% confluence. Treat cells by adding fresh media containing regulator for desired time.
2. Remove media and rinse cells once with ice-cold 1X PBS.
3. 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.
4. Scrape cells off the plate and transfer to an appropriate tube. Keep on ice.
5. Sonicate lysates on ice.
6. Microcentrifuge for 10 min (x14,000 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

1. Remove media by low speed centrifugation (~1200 rpm) when the culture reaches 0.5–1.0 × 10⁶ viable cells/ml. Treat cells by adding fresh media containing regulator for desired time.
2. Collect cells by low speed centrifugation (~1200 rpm) and wash once with 5–10 ml ice-cold 1X PBS.
3. Cells harvested from 50 ml of growth media can be lysed in 2.0 ml of 1X Cell Lysis Buffer plus 1 mM PMSF.
4. Sonicate lysates on ice.
5. Microcentrifuge for 10 min (x14,000 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

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. 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.
 3. Add 100 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 37°C. Alternatively, the plate can be incubated overnight at 4°C.
 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 reconstituted Detection Antibody (green color) to each well (refer to Section A, Step 2). Seal with tape and incubate the plate at 37°C for 1 hr.
 6. Repeat wash procedure (Section C, Step 4).
 7. Add 100 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 for 30 min at 37°C.
 8. Repeat wash procedure (Section C, Step 4).
 9. Add 100 µl of TMB Substrate to each well. Seal with tape and incubate the plate for 10 min at 37°C or 30 min 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 min after adding STOP Solution.
 - b. **Spectrophotometric Determination:** Wipe underside of wells with a lint-free tissue. Read absorbance at 450 nm within 30 min after adding STOP Solution.