

#7297 Store at 4°C

PathScan® Total EGF Receptor Chemiluminescent Sandwich ELISA Kit

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Species Cross Reactivity
H

UniProt ID:
#P00533

Entrez-Gene Id:
#1956



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Product Includes	Product #	Quantity	Color	Storage Temp
EGF Receptor Rabbit Detection mAb	12951	1 ea	Green (Lyophilized)	4°C
Anti-rabbit IgG, HRP-linked Antibody (ELISA Formulated)	13272	1 ea	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	Colorless	RT
Stable Peroxide Buffer	42552	3 ml	Colorless	RT
Sealing Tape	54503	2 ea		4°C
ELISA Wash Buffer (20X)	9801	25 ml	Colorless	4°C
ELISA Sample Diluent	11083	25 ml	Blue	4°C
Cell Lysis Buffer (10X)	9803	15 ml	Yellowish	-20°C

*The microwell plate is supplied as 12 8-well modules - Each module is designed to break apart for 8 tests.

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 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 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).

Background References

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- 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.
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Cross-Reactivity Key

H: human **M:** mouse **R:** rat **Hm:** hamster **Mk:** monkey **Vir:** virus **Mi:** mink **C:** chicken **Dm:** D. melanogaster
X: Xenopus **Z:** zebrafish **B:** bovine **Dg:** dog **Pg:** pig **Sc:** S. cerevisiae **Ce:** C. elegans **Hr:** horse
GP: Guinea Pig **Rab:** rabbit **All:** all species expected

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#7297



PathScan® Total EGF Receptor Chemiluminescent Sandwich ELISA Kit

ELISA Chemiluminescent

NOTE: Refer to product-specific datasheets or product webpage for assay incubation temperature. This /product/productDetail.jsp?productId=luminescent ELISA is offered in low volume microplate. Samples and reagents only require 50 µl per microwell.

A. Solutions and Reagents

NOTE: Prepare solutions with reverse osmosis deionized (RODI) or equivalent grade water.

1. **20X Phosphate Buffered Saline (PBS):** (#9808) To prepare 1 L 1X PBS: add 50 ml 10X PBS to 950 ml dH₂O, mix.
2. Bring all microwell strips to room temperature before use.
3. Prepare 1X wash buffer by diluting 20X Wash Buffer (included in each PathScan® Sandwich ELISA Kit) in dH₂O.
4. **1X Cell Lysis Buffer:** 10X Cell Lysis Buffer (#9803): To prepare 10 ml of 1X Cell Lysis Buffer, add 1 ml of 10X Cell Lysis Buffer to 9 ml of dH₂O, mix. Buffer can be stored at 4°C for short-term use (1–2 weeks).

Recommended: Add 1 mM phenylmethylsulfonyl fluoride (PMSF) (#8553) immediately before use.

5. **20X LumiGLO® Reagent and 20X Peroxide:** (#7003).

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 (~1,200 rpm) when the culture reaches 0.5–1.0 x 10⁶ viable cells/ml. Treat cells by adding fresh media containing regulator for desired time.
2. Collect cells by low speed centrifugation (~1,200 rpm) and wash once with 5–10 ml ice-cold 1X PBS.
3. Cells harvested from 50 ml of growth medium 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 in the storage bag and stored at 4°C immediately.
2. Cell lysates can be used undiluted or diluted with sample diluent (supplied in each PathScan® Sandwich ELISA Kit, blue color). Individual datasheets or product webpage for each kit provide information regarding an appropriate dilution factor for lysates and kit assay results.
3. 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.
4. **Gently remove the tape and wash wells:**
 1. Discard plate contents into a receptacle.
 2. Wash 4 times with 1X Wash Buffer, 150 µl each time per well.
 3. For each wash, strike plates on fresh paper towels hard enough to remove the residual solution in each well, but do not allow wells to dry completely at any time.
 4. Clean the underside of all wells with a lint-free tissue.
5. Add 50 µl of detection antibody (green color) to each well. Seal with tape and incubate the plate at room temperature for 1 hr.
6. Repeat wash procedure (Section C, Step 4).
7. Add 50 µl of HRP-linked secondary antibody (red color) to each well. Seal with tape and incubate the plate at room temperature for 30 min.
8. Repeat wash procedure (Section C, Step 4).
9. Prepare detection reagent working solution by mixing equal parts 2X LumiGLO® Reagent and 2X Peroxide.
10. Add 50 µl of the detection reagent working solution to each well.
11. Use a plate-based luminometer set at 425 nm to measure Relative Light Units (RLU) within 1–10 min following addition of the substrate.
 1. Optimal signal intensity is achieved when read within 10 min.

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