Store at 4°C and -20°C

#8951

PathScan® EGF Receptor (L858R Mutant Specific) Sandwich ELISA Kit

☑ 1 Kit
(96 assays)

Cell Signaling TECHNOLOGY®

Orders ■ 877-616-CELL (2355)
orders@cellsignal.com
Support ■ 877-678-TECH (8324)
info@cellsignal.com
Web ■ www.cellsignal.com

rev. 03/21/14

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

Entrez-Gene ID #1956
UniProt ID #P00533

Species Cross-Reactivity: H

Description: The PathScan® EGF Receptor (L858R Mutant Specific) Sandwich ELISA Kit is a solid phase sandwich enzyme-linked immunosorbent assay (ELISA) that detects endogenous levels of EGF receptor L858R mutant protein. An EGF receptor (L858R Mutant Specific) rabbit mAb has been coated on the microwells. After incubation with cell lysates, EGF receptor L858R mutant protein is captured by the coated antibody. Following extensive washing, an EGF receptor mouse monoclonal detection antibody is added to detect captured EGF receptor mutant protein. Anti-mouse IgG, HRP-linked antibody is then used to recognize the bound detection antibody. HRP substrate, TMB, is added to develop color. The magnitude of the absorbance for this developed color is proportional to the quantity of EGF receptor L858R mutant protein.

Antibodies in kit are custom formulations specific to kit.

Background: The epidermal growth factor (EGF) receptor is a 170 kDa transmembrane tyrosine kinase that belongs to the HER/ErbB protein family. Research studies have shown that somatic mutations in the tyrosine kinase domain of EGF receptor (EGFR) are present in a subset of lung adenocarinomas that respond to EGFR inhibitors, such as gefinitib and erlotinib (1-3). Two types of mutations account for approximately 90% of mutated cases: a specific point mutation, L858R, that occurs in exon 21 and short in-frame deletions in exon 19 (4,5). The most frequent exon 19 deletion is E746-A750, accounting for 90% of lesions at this site, although some rare variants occur.

Specificity/Sensitivity: PathScan® EGF Receptor (L858R Mutant Specific) Sandwich ELISA Kit #8951 detects endogenous levels of EGF Receptor L858R mutant protein in human cells, as shown in Figure 1. The kit sensitivity is shown in Figure 2. This kit detects proteins from the indicated species, as determined through in-house testing, but may also detect homologous proteins from other species.

Background References:

(1) Lynch, T.J. et al. (2004) *N Engl J Med* 350, 2129-39.
(2) Pao, W. et al. (2004) *Proc Natl Acad Sci U S A* 101, 13306-11.
(3) Haber, D.A. et al. (2005) *Cold Spring Harb Symp Quant Biol* 70, 419-26.
(4) Kosaka, T. et al. (2004) *Cancer Res* 64, 8919-23.
(5) Riely, G.J. et al. (2006) *Clin Cancer Res* 12, 7232-41.

Products Included	Volume	Solution Color
EGF Receptor (L858R Mutant Specific) Rabbit mAb Coated Microwells*	96 tests	
EGF Receptor Mouse Detection Antibody	11 ml	green
Anti-mouse IgG, HRP-linked Antibody	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).

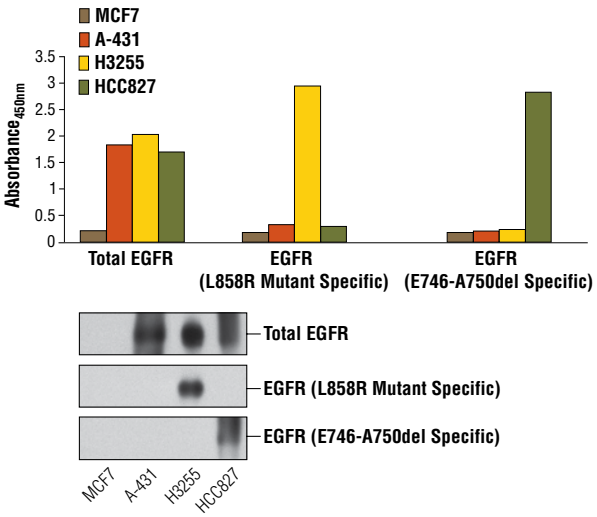

Figure 1. EGFR mutant protein from H3255 cells can be detected by PathScan® EGF Receptor (L858R Mutant Specific) Sandwich ELISA Kit #8951. However, this kit cannot detect wild type EGFR protein from A-431 cells or EGFR E746-A750del protein from HCC827 cells. EGFR (either wild type or mutant) from these three cell lines can be detected by PathScan® Total EGF Receptor Sandwich ELISA Kit #7250, but no detection is found in MCF7 cells, which lack expression of EGFR. The absorbance readings at 450 nm are shown in the top figure, while the corresponding western blots using EGF Receptor (L858R Mutant Specific) (43B2) Rabbit mAb #3197, EGF Receptor (E746-A750del Specific) (6B6) XP® Rabbit mAb #2085, or EGF Receptor (C74B9) Rabbit mAb #2646 are shown in the bottom figure.

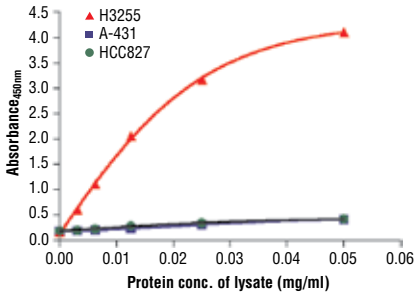

Figure 2. The relationship between protein concentration of lysates from A-431, H3255, or HCC827 cells and the absorbance at 450 nm is shown. A-431, H3255, or HCC827 cells (85% confluence) were harvested and then lysed.

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

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**—zebrafish **B**—bovine **Dg**—dog **Pg**—pig **Sc**—S. cerevisiae **Ce**—C. elegans **Hr**—horse **All**—all species expected Species enclosed in parentheses are predicted to react based on 100% homology.

#8951

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, 1µg/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 phenylmethylsulfonyl 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 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. Generally, 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. However, dilution factors need to be titrated when specific cell lysates are used.
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 4.
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 4.
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.