



Orders: 877-616-CELL (2355)
orders@cellsignal.com

Support: 877-678-TECH (8324)

Web: info@cellsignal.com
cellsignal.com

3 Trask Lane | Danvers | Massachusetts | 01923 | USA

Store at +4C
#7702

PathScan® Phospho-EGF Receptor (Tyr1068) (E746-A750del Specific) Sandwich ELISA Kit

Species Cross Reactivity: H UniProt ID: #P00533 Entrez-Gene Id: #1956

For Research Use Only. Not for Use in Diagnostic Procedures.

| Product Includes | Product # | Quantity | Color | Storage Temp |
|-------------------------|-----------|----------|-----------|--------------|
| TMB Substrate | 7004 | 11 ml | Colorless | +4C |
| STOP Solution | 7002 | 11 ml | Colorless | +4C |
| Sealing Tape | 54503 | 2 ea | | +4C |
| ELISA Wash Buffer (20X) | 9801 | 25 ml | Colorless | +4C |
| ELISA Sample Diluent | 11083 | 25 ml | Blue | +4C |
| Cell Lysis Buffer (10X) | 9803 | 15 ml | Yellowish | -20C |

Kit contents scale proportionally with size, except sealing tape.

Example: The V1 kit contains 5X the listed quantities above, but will exclude the sealing tape.

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

Description

The PathScan® Phospho-EGF Receptor (Tyr1068) (E746-A750del Specific) Sandwich ELISA Kit is a solid phase sandwich enzyme-linked immunosorbent assay (ELISA) that detects endogenous levels of Phospho-EGF receptor (Tyr1068) (E746-A750del) mutant protein. An EGF Receptor (E746-A750del Specific) Rabbit Antibody has been coated on the microwells. After incubation with cell lysates, EGF receptor (E746-A750del) mutant protein (phospho and nonphospho) is captured by the coated antibody. Following extensive washing, a Phospho-EGF Receptor (Tyr1068) Mouse Detection Antibody is added to detect captured phosphorylated EGF receptor (E746-A750del) 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 phospho-EGF receptor (Tyr1068) (E746-A750del) mutant protein. Antibodies in kit are custom formulations specific to kit.

Specificity/Sensitivity

PathScan® Phospho-EGF Receptor (Tyr1068) (E746-A750del Specific) Sandwich ELISA Kit #7702 detects endogenous levels of phospho-EGF receptor (Tyr1068) (E746-A750del) 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

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 adenocarcinomas that respond to EGFR inhibitors, such as gefitinib 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.

Background References

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

Trademarks and Patents

Cell Signaling Technology is a trademark of Cell Signaling Technology, Inc.

PathScan is a registered trademark of Cell Signaling Technology, Inc.

All other trademarks are the property of their respective owners. Visit cellsignal.com/trademarks for more information.

Limited Uses

Except as otherwise expressly agreed in a writing signed by a legally authorized representative of CST, the following terms apply to Products provided by CST, its affiliates or its distributors. Any Customer's terms and conditions that are in addition to, or different from, those contained herein, unless separately accepted in writing by a legally authorized representative of CST, are rejected and are of no force or effect.

Products are labeled with For Research Use Only or a similar labeling statement and have not been approved, cleared, or licensed by the FDA or other regulatory foreign or domestic entity, for any purpose. Customer shall not use any Product for any diagnostic or therapeutic purpose, or otherwise in any manner that conflicts with its labeling statement. Products sold or licensed by CST are provided for Customer as the end-user and solely for research and development uses. Any use of Product for diagnostic, prophylactic or therapeutic purposes, or any purchase of Product for resale (alone or as a component) or other commercial purpose, requires a separate license from CST. Customer shall (a) not sell, license, loan, donate or otherwise transfer or make available any Product to any third party, whether alone or in combination with other materials, or use the Products to manufacture any commercial products, (b) not copy, modify, reverse engineer, decompile, disassemble or otherwise attempt to discover the underlying structure or technology of the Products, or use the Products for the purpose of developing any products or services that would compete with CST products or services, (c) not alter or remove from the Products any trademarks, trade names, logos, patent or copyright notices or markings, (d) use the Products solely in accordance with CST Product Terms of Sale and any applicable documentation, and (e) comply with any license, terms of service or similar agreement with respect to any third party products or services used by Customer in connection with the Products.

#7702

PathScan[®] Phospho-EGF Receptor (Tyr1068) (E746-A750del Specific) Sandwich ELISA Kit

ELISA Colorimetric

NOTE: Refer to product-specific datasheets or product webpage for assay incubation temperature.

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 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.

NOTE: Refer to product-specific datasheet or webpage for lysis buffer recommendation.

5. **TMB Substrate:** (#7004).
6. **STOP Solution:** (#7002).

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 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 in the storage bag 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 or product webpage for each kit provide information regarding an appropriate dilution factor for lysates and kit assay results.
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:**
 1. Discard plate contents into a receptacle.
 2. Wash 4 times with 1X wash buffer, 200 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 completely dry at any time.
 4. Clean the underside of all wells with a lint-free tissue.

5. Add 100 μ l of detection antibody (green color) to each well. Seal with tape and incubate the plate at 37°C for 1 hr.
6. Repeat wash procedure (Section C, Step 4).
7. Add 100 μ l of HRP-linked secondary antibody (red color) to each well. Seal with tape and incubate the plate for 30 min at 37°C.
8. Repeat wash procedure (Section C, Step 4).
9. Add 100 μ l 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 μ l 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**

1. **Visual Determination:** Read within 30 min after adding STOP solution.
2. **Spectrophotometric Determination:** Wipe underside of wells with a lint-free tissue. Read absorbance at 450 nm within 30 min after adding STOP solution.

posted June 2005

revised November 2013