

PathScan® Total eIF4E Sandwich ELISA Kit



Cell Signaling
TECHNOLOGY®

✓ 1 Kit
(96 assays)

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This product is for *in vitro* research use only and is not intended for use in humans or animals.
This product is not intended for use as a therapeutic or in diagnostic procedures.

Entrez-Gene ID #1977
Swiss-Prot Acc. #P06730

Species Cross-Reactivity: H

Introduction: The PathScan® Total eIF4E Sandwich ELISA Kit is a solid phase sandwich enzyme-linked immunosorbent assay (ELISA) that detects endogenous levels of eIF4E. An eIF4E mouse antibody has been coated onto the microwells. After incubation with cell lysates, eIF4E (phospho and nonphospho) is captured by the coated antibody. Following extensive washing, a eIF4E rabbit detection antibody is added to the captured phospho and nonphospho eIF4E protein. Anti-rabbit IgG, HRP-linked Antibody #7074 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 total eIF4E.

Companion Products:

eIF4E Antibody #9742
Phospho-eIF4E (Ser209) Antibody #9741
eIF4E (C46H6) Rabbit mAb #2067
Cell Lysis Buffer (10X) #9803
Phosphate Buffered Saline (PBS-20X) #9808
Phosphate Buffered Saline with Tween 20 (PBST-20X) #9809
BSA #9998
TMB Substrate #7004
STOP Solution #7002

Specificity/Sensitivity: CST's PathScan® Total eIF4E Sandwich ELISA Kit detects endogenous levels of total eIF4E protein. As shown in Figure 1, a significant induction of eIF4E phosphorylation at Ser209 can be detected in HeLa cells following treatment with 20% FBS using the Phospho-eIF4E (Ser209) Sandwich ELISA Kit #7938. The levels of total eIF4E remain unchanged as shown by western analysis and by PathScan® Total eIF4E Sandwich ELISA Kit #7940 (Figure 1).

| Products Included | Volume | Solution Color |
|--------------------------------------|----------|----------------|
| eIF4E Antibody Coated Microwells* | 96 tests | |
| eIF4E Detection Antibody | 11 ml | green |
| Anti-rabbit 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).

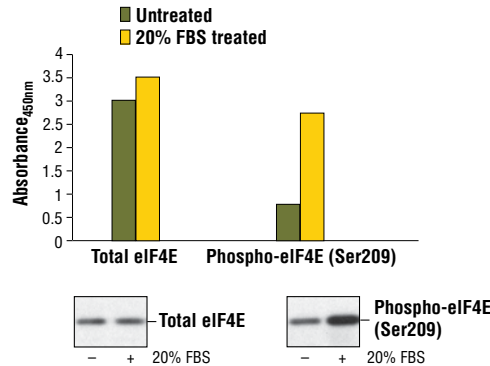


Figure 1. Treatment of serum-starved HeLa cells with 20% FBS stimulates phosphorylation of eIF4E at Ser209, detected by the PathScan® Phospho-eIF4E (Ser209) Sandwich ELISA Kit #7938, but does not affect the levels of total eIF4E detected by PathScan® Total eIF4E Sandwich ELISA Kit #7940. HeLa cells (80-90% confluent) were serum-starved over night and then treated with 20% FBS at 37°C. The absorbance readings at 450 nm are shown in the top figure, while the corresponding western blots using eIF4E Antibody #9742 (left panel) or Phospho-eIF4E (Ser209) Antibody #9741 (right panel) are shown in the bottom figure.

Background: Eukaryotic initiation factor 4E (eIF4E) binds to the mRNA cap structure to mediate the initiation of translation (1,2). eIF4E interacts with eIF4G, a scaffold protein that promotes assembly of eIF4E and eIF4A into the eIF4F complex (2). eIF4B is thought to assist the eIF4F complex in translation initiation. Upon activation by mitogenic and/or stress stimuli mediated by Erk and p38 MAPK, Mnk1 phosphorylates eIF4E at Ser209 *in vivo* (3,4). Two Erk and p38 MAPK phosphorylation sites in mouse Mnk1 (Thr197 and Thr202) are essential for Mnk1 kinase activity (3). The carboxy-terminal region of eIF4G also contains serum-stimulated phosphorylation sites, including Ser1108, Ser1148 and Ser1192 (5). Phosphorylation at these sites is blocked by the PI3 kinase inhibitor LY294002 and by the FRAP/mTOR inhibitor rapamycin.

Background References:

- (1) Sonenberg, N. et al. (1978) *Proc. Natl. Acad. Sci. USA* 75, 4843–4847.
- (2) Gingras, A.C. et al. (1999) *Annu. Rev. Biochem.* 68, 913–963.
- (3) Waskiewicz, A. et al. (1999) *Mol. Cell. Biol.* 19, 1871–1880.
- (4) Pyronnet, S. et al. (1999) *EMBO J.* 18, 270–279.
- (5) Raught, B. et al. (2000) *EMBO J.* 19, 434–444.

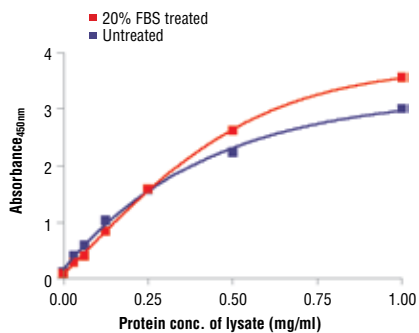


Figure 2. The relationship between the protein concentration of lysates from untreated and 20% FBS-treated HeLa cells and the absorbance at 450 nm using the PathScan® Total eIF4E Sandwich ELISA Kit is shown.

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 Na₃VO₄, 1 μg/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 phenyl-methylsulfonyl 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.