

PathScan® Phospho-4E-BP1 (Thr37/Thr46) Sandwich ELISA Kit

✓ 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.

Species Cross-Reactivity: H, M, R, Mk

Introduction: CST's PathScan® Phospho-4E-BP1 (Thr37/46) Sandwich ELISA Kit is a solid phase sandwich enzyme-linked immunosorbent assay (ELISA) that detects endogenous levels of 4E-BP1 when phosphorylated at Thr37/46. A Phospho-4E-BP1 (Thr37/46) Rabbit Antibody* has been coated onto the microwells. After incubation with cell lysates, phospho-4E-BP1 (Thr37/46) is captured by the coated antibody. Following extensive washing, a 4E-BP1 Mouse Detection Antibody* is added to detect the captured phospho-4E-BP1 protein. Anti-mouse IgG, HRP-linked Antibody #7076* is then used to recognize the bound detection antibody. HRP substrate, TMB, is added to develop color. The magnitude of absorbance for this developed color is proportional to the quantity of 4E-BP1 phosphorylated at Thr37/46.

* Antibodies in kit are custom formulations specific to kit.

Companion Products:

Phospho-4E-BP1 (Thr37/46) Antibody #9459
Anti-mouse IgG, HRP-linked Antibody #7076
4E-BP1 (53H11) Rabbit mAb #9644
4E-BP1 Antibody #9452
Phospho-4E-BP1 (Ser65) (174A9) Rabbit mAb #9456
Phospho-4E-BP1 (Ser65) Antibody #9451
Phospho-4E-BP1 (Thr37/46) (236B4) Rabbit mAb #2855
Phospho-4E-BP1 (Thr70) Antibody #9455
Nonphospho-4E-BP1 (Thr46) (87D12) Rabbit mAb #4923
4E-BP2 Antibody #2845
Cell Lysis Buffer (10X) #9803

Specificity/Sensitivity: CST's PathScan® Phospho-4E-BP1 (Thr37/46) Sandwich ELISA Kit detects endogenous levels of phospho-4E-BP1 when phosphorylated at Thr37/46. As shown in Figure 1, using the Phospho-4E-BP1 (Thr37/46) ELISA Kit #7216, a significant induction of 4E-BP1 phosphorylation at Thr37/46 is detected in serum and amino acid starved HEK-293T cells treated with insulin for 30 minutes after replenishing the amino acids.

Background: When bound to eIF4E, 4E-BP1 (also known as PHAS-1) inhibits cap-dependent translation. Upon hyperphosphorylation of 4E-BP1 this interaction is disrupted and cap-dependent translation is activated (1). Both the PI3 kinase/Akt pathway and FRAP/mTOR kinase regulate 4E-BP1 activity (2,3). Multiple 4E-BP1 residues are phosphorylated *in vivo* (4); while phosphorylation by FRAP/mTOR on Thr37 and Thr46 does not prevent the binding of 4E-BP1 to eIF4E, it is thought to prime 4E-BP1 for subsequent phosphorylation at Ser65 and Thr70 (5).

Applications Key: W—Western IP—Immunoprecipitation IHC—Immunohistochemistry IC—Immunocytochemistry IF—Immunofluorescence F—Flow cytometry E—ELISA D—DELFIATM

Species Cross-Reactivity Key: H—human M—mouse R—rat Hm—hamster Mk—monkey Mi—mink C—chicken X—Xenopus Z—zebra fish B—bovine All—all species expected
Species enclosed in parentheses are predicted to react based on 100% sequence homology.

Products Included	Volume	Solution Color
4E-BP1 (Thr37/46) Rabbit Antibody Coated Microwells	96 tests	
4E-BP1 Mouse Detection Antibody	11 ml	green
Anti-Mouse IgG HRP-Linked Ab	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).

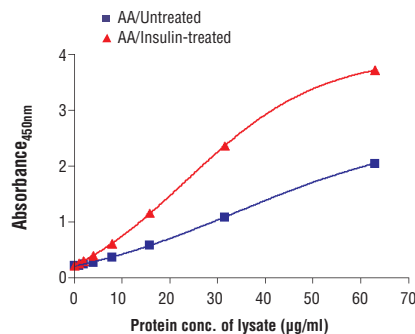


Figure 2: The relationship between the protein concentration of lysate from amino acid (AA)/untreated and AA/insulin-treated HEK-293T and the absorbance at 450 nm is shown.

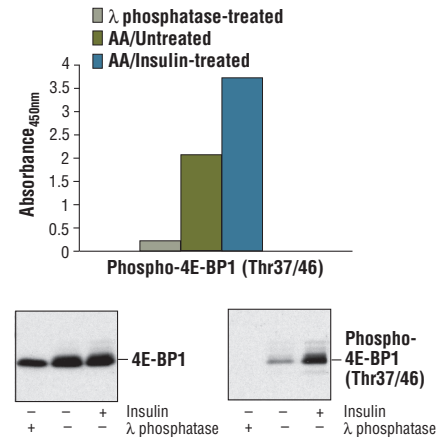


Figure 1: Treatment of HEK-293T cells with amino acids and insulin stimulates phosphorylation of 4E-BP1 at Thr37/46, as detected by PathScan® Phospho-4E-BP1 (Thr37/46) Sandwich ELISA Kit #7216, but does not affect the level of total 4E-BP1 protein detected by Western analysis. HEK-293T cells (70–80% confluent) were starved overnight and deprived of amino acids for 1 hour. The amino acids were replenished for 1 hour. Cells were either untreated or stimulated with 100 nM insulin for 30 minutes at 37°C. λ phosphatase treatment of control cell lysates (4000 U/mL for 60 minutes at 37°C) abolishes the basal phosphorylation of 4E-BP1 as shown by both sandwich ELISA and Western analysis. The absorbance readings at 450 nm are shown in the top figure, while the corresponding Western blots, using 4E-BP1 Antibody #9452 (left panel) or Phospho-4E-BP1 (Thr37/46) Antibody #9459 (right panel), are shown in the bottom figure.

Background References:

- (1) Pause, A. et al. (1994) *Nature* 371, 762–767.
- (2) Brunn, G.J. et al. (1997) *Science* 277, 99–101.
- (3) Gingras, A.C. et al. (1998) *Genes Dev.* 12, 502–513.
- (4) Fadden, P. et al. (1997) *J. Biol. Chem.* 272, 10240–10247.
- (5) Gingras, A.C. et al. (1999) *Genes Dev.* 13, 1422–1437.

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 cm²) 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. (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.

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 C4.
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 C4.
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.