

PathScan® Phospho-ATF-2 (Thr71) 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.

Species Cross-Reactivity: H, M

Introduction: CST's PathScan® Phospho-ATF-2 (Thr71) Sandwich ELISA Kit is a solid phase sandwich enzyme-linked immunosorbent assay (ELISA) that detects endogenous levels of phospho-ATF-2 (Thr71) protein. A Phospho-ATF-2 (Thr 71) Antibody has been coated onto the microwells. After incubation with cell lysates, phospho-ATF-2 protein is captured by the coated antibody. Following extensive washing, a total ATF-2 Mouse mAb is added to detect the captured phospho-ATF-2 protein. HRP-linked Anti-mouse Antibody #7076* is then used to recognize the bound detection antibody. HRP substrate, TMB, is added to develop color. The magnitude of optical density for this developed color is proportional to the quantity of phospho-ATF-2 (Thr71).

* Antibodies in this kit are custom formulations specific to the kit.

Companion Products:

Phospho-ATF-2 (Thr71) Antibody #9221

Phospho-ATF-2 (Thr69/71) Antibody #9225

ATF-2 (20F1) Rabbit mAb #9226

Anti-mouse IgG, HRP-linked Antibody #7076

Cell Lysis Buffer (10X) #9803

Specificity/Sensitivity: CST's PathScan® Phospho-ATF-2 (Thr71) Sandwich ELISA Kit detects endogenous levels of Phospho-ATF-2 protein. Using this Sandwich ELISA Kit #7185, a significant induction of phospho-ATF-2 in NIH/3T3 cells treated with anisomycin can be detected. However, the level of total ATF-2 (phospho- and nonphospho-), detected by PathScan® Total ATF-2 Sandwich ELISA Kit #7195, remains unchanged (Figure 1). This kit can also be used to detect phosphorylated ATF-2 protein in human HeLa and 293 cells.

Products Included	Volume	Solution Color
ATF-2 (Thr71) Antibody Coated Microwells*	96 tests	
ATF-2 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).

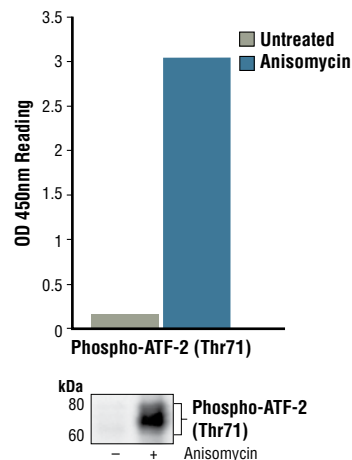


Figure 1: Treatment of NIH/3T3 cells with anisomycin stimulates phosphorylation of ATF-2 at Thr71, detected by PathScan® Phospho-ATF-2 (Thr71) Sandwich ELISA kit #7185, but does not affect the level of total ATF-2 protein detected by PathScan® Total ATF-2 ELISA kit #7195. OD 450 readings are shown in the top portion of the figure, while the corresponding Western blot using ATF-2 Rabbit mAb #9226 (left panel) or phospho-ATF-2 (Thr71) Antibody #9221 (right panel) is shown in the bottom portion of the figure.



Background: The transcription factor ATF-2 (also called CRE-BP1) binds to both AP-1 and CRE DNA response elements and is a member of the ATF/CREB family of leucine zipper proteins (1). ATF-2 interacts with a variety of viral oncoproteins and cellular tumor suppressors and is a target of the SAPK/JNK and p38 MAP kinase signaling pathways (2-4). Various forms of cellular stress, including genotoxic agents, inflammatory cytokines and UV irradiation, stimulate the transcriptional activity of ATF-2. Cellular stress activates ATF-2 by phosphorylation of Thr69 and Thr71 (2-4). Both SAPK and p38 MAPK have been shown to phosphorylate ATF-2 at these sites *in vitro* and in cells transfected with ATF-2. Mutations of these sites result in the loss of stress-induced transcription by ATF-2 (2-4). In addition, mutations at these sites reduce the ability of E1A and Rb to stimulate gene expression via ATF-2 (2).

Background References:

- (1) Abdel-Hafiz, H.A. et al. (1992) *Mol. Endocrinol.* 6, 2079–2089.
- (2) Gupta, S. et al. (1995) *Science* 267, 389–393.
- (3) van Dam, H. et al. (1995) *EMBO J.* 14, 1798–1811.
- (4) Livingstone, C. et al. (1995) *EMBO J.* 14, 1785–1797.

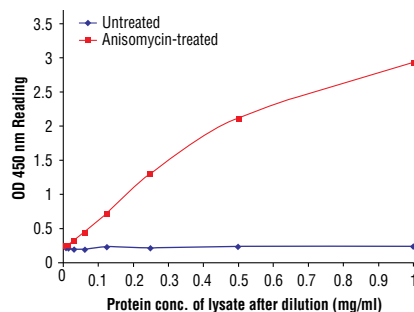


Figure 2: Relationship between protein concentration of lysates from untreated and anisomycin-treated NIH/3T3 cells and kit assay optical density readings. NIH/3T3 cells (80% confluence) were treated with anisomycin and lysed after incubation at 37°C for 30 minutes.

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.