#11874 Store at 4°C

PathScan® Phospho-Bcl-2 (Ser70) Sandwich ELISA Kit

☑ 1 Kit
(96 assays)

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

Entrez-Gene ID #596
UniProt ID #P10415

Species Cross-Reactivity: H

Description: The PathScan® Phospho-Bcl-2 (Ser70) Sandwich ELISA Kit is a solid phase sandwich enzyme-linked immunosorbent assay (ELISA) that detects endogenous levels of Bcl-2 when phosphorylated at Ser70. A Phospho-Bcl-2 (Ser70) Rabbit mAb has been coated onto the microwells. After incubation with cell lysates, phosphorylated Bcl-2 protein is captured by the coated antibody. Following extensive washing, a Bcl-2 Mouse Detection mAb is added to detect the captured phospho-Bcl-2 (Ser70) 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 the developed color is proportional to the quantity of Bcl-2 phosphorylated at Ser70.

Antibodies in kit are custom formulations specific to kit.

Specificity/Sensitivity: The PathScan® Phospho-Bcl-2 (Ser70) Sandwich ELISA Kit recognizes endogenous levels of Bcl-2 protein when phosphorylated at Ser70 as shown in Figure 1. 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: Bcl-2 exerts a survival function in response to a wide range of apoptotic stimuli through inhibition of mitochondrial cytochrome c release (1). It has been implicated in modulating mitochondrial calcium homeostasis and proton flux (2). Several phosphorylation sites have been identified within Bcl-2 including Thr56, Ser70, Thr74, and Ser87 (3). It has been suggested that these phosphorylation sites may be targets of the ASK1/MKK7/JNK1 pathway and that phosphorylation of Bcl-2 may be a marker for mitotic events (4,5). Mutation of Bcl-2 at Thr56 or Ser87 inhibits its anti-apoptotic activity during glucocorticoid-induced apoptosis of T lymphocytes (6). Interleukin-3 and JNK-induced Bcl-2 phosphorylation at Ser70 may be required for its enhanced anti-apoptotic functions (7).

Background References:

(1) Murphy, K.M. et al. (2000) *Cell Death Differ* 7, 102-11.
(2) Zhu, L. et al. (1999) *J Biol Chem* 274, 33267-73.
(3) Maundrell, K. et al. (1997) *J Biol Chem* 272, 25238-42.
(4) Yamamoto, K. et al. (1999) *Mol Cell Biol* 19, 8469-78.
(5) Ling, Y.H. et al. (1998) *J Biol Chem* 273, 18984-91.
(6) Huang, S.T. and Cidlowski, J.A. (2002) *FASEB J* 16, 825-32.
(7) Deng, X. et al. (2001) *J Biol Chem* 276, 23681-8.

Products Included	Volume	Solution Color
Phospho-Bcl-2 (Ser70) Rabbit mAb Coated Microwells*	96 tests	
Bcl-2 Mouse Detection mAb	11 ml	green
Anti-mouse IgG, HRP-linked Antibody #7076	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).

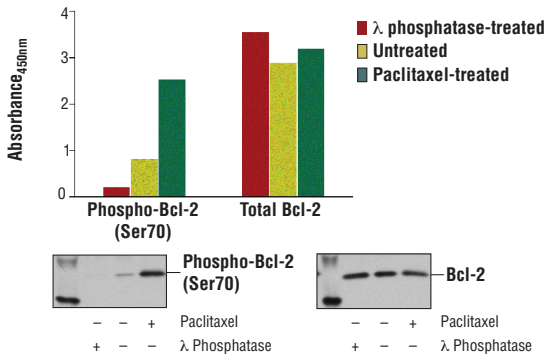
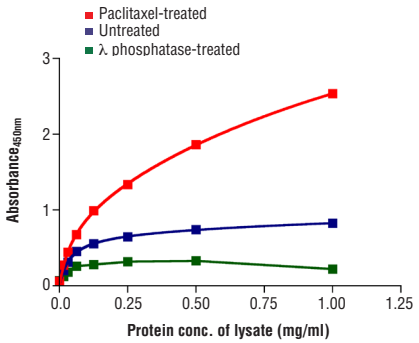


Figure 1. Treatment of Jurkat cells with Paclitaxel stimulates phosphorylation of Bcl-2 at Ser70, as detected by the PathScan® Phospho-Bcl-2 (Ser70) Sandwich ELISA Kit, but does not affect the levels of total Bcl-2 detected by PathScan® Total Bcl-2 Sandwich ELISA Kit #12030. Jurkat cells were untreated or treated with λ phosphatase treated or Paclitaxel #9807 (1 mM, 20 hr, 37°C). The absorbance readings at 450 nm are shown in the top figure, while the corresponding western blots using Phospho-Bcl-2 (Ser70) (5H2) Rabbit mAb #2827 (left panel) or Bcl-2 (D55G8) Rabbit mAb (Human Specific) #4223 (right panel) are shown in the bottom figure.



◀ *Figure 2. The relationship between protein concentration of lysate from Jurkat cells, untreated or treated with λ phosphatase or Paclitaxel #9807 (1 mM, 20 hr, 37°C), and the absorbance at 450 nm is shown.*

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.

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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.