PathScan® Total Bad Sandwich ELISA Kit

#7162C Store at 4°C

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✓ 1 Kit
(96 assays)

rev. 08/01/17

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

Species Cross-Reactivity: H, Mk

Description: CST's PathScan® Total Bad Sandwich ELISA Kit is a solid phase sandwich enzyme-linked immunosorbent assay (ELISA) that detects endogenous levels of total Bad protein. A Bad rabbit mAb* has been coated onto the microwells. After incubation with cell lysates, Bad protein (phospho and nonphospho) is captured by the coated antibody. Following extensive washing, a Bad mouse mAb* is added to detect the captured Bad 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 total bad protein.

* Antibodies in kit are custom formulations specific to kit.

Specificity/Sensitivity: CST's PathScan® Total Bad Sandwich ELISA Kit #7162 detects endogenous levels of Bad protein. A significant induction of Bad phosphorylation at Ser112 can be detected in TPA-treated OVCAR8 cells using PathScan® Phospho-Bad (Ser112) Sandwich ELISA Kit #7182. However, the level of total Bad protein (phospho and nonphospho) detected by PathScan® Total Bad Sandwich ELISA Kit #7162 remains unchanged (Figure 1). In Figure 3, Western analysis of protein captured in microwells coated with the Bad antibody shows a major band corresponding to the Bad protein.

Background: Bad is a proapoptotic member of the Bcl-2 family that can displace Bax from binding to Bcl-2 and Bcl-xL, resulting in cell death (1,2). Survival factors such as IL-3 can inhibit the apoptotic activity of Bad by activating intracellular signaling pathways that result in the phosphorylation of Bad at Ser112 and Ser136 (2). Phosphorylation at these sites results in the binding of Bad to 14-3-3 proteins and the inhibition of Bad binding to Bcl-2 and Bcl-xL (2). Akt has been shown to promote cell survival via its ability to phosphorylate Bad at Ser136 (3,4). Bad is phosphorylated at Ser112 both *in vivo* and *in vitro* by p90RSK (5,6) and mitochondria-anchored PKA (7). Phosphorylation of Ser155 in the BH3 domain by PKA plays a critical role in blocking the dimerization of Bad and Bcl-xL (8-10).

Product Includes	Item #	Kit Quantity	Color	Storage Temp
Bad Rabbit mAb Coated Microwells*	39513	96 tests		4°C
Bad Mouse Detection mAb	9254	1 each	Green (Lyophilized)	4°C
Anti-mouse IgG, HRP-linked Antibody (ELISA Formulated)	13304	1 each	Red (Lyophilized)	4°C
Detection Antibody Diluent	13339	11 ml	Green	4°C
HRP Diluent	13515	11 ml	Red	4°C
TMB Substrate	7004	11 ml		4°C
STOP Solution	7002	11 ml		4°C
Sealing Tape	54503	2 sheets		4°C
ELISA Wash Buffer (20X)	9801	25 ml		4°C
ELISA Sample Diluent	11083	25 ml	Blue	4°C
Cell Lysis Buffer (10X)	9803	15 ml		-20°C

*12 8-well modules – Each module is designed to break apart for 8 tests.

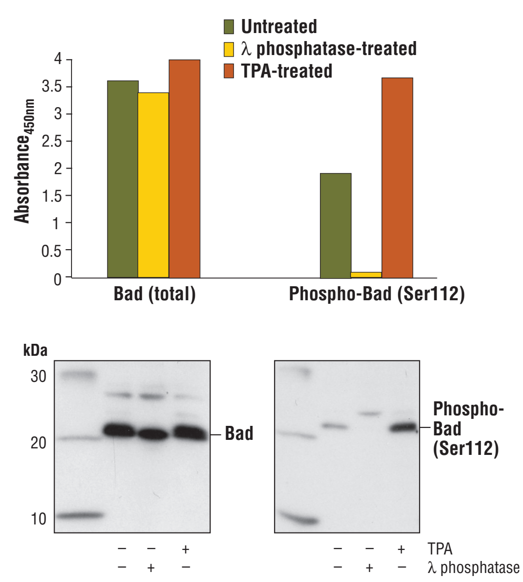

Figure 1. Treatment of OVCAR8 cells with TPA stimulates phosphorylation of Bad at Ser112, detected by PathScan® Phospho-Bad (Ser112) Sandwich ELISA Kit #7182, but does not affect the level of total Bad protein detected by PathScan® Total Bad Sandwich ELISA Kit #7162. λ phosphatase treatment of control cell lysates (4000 U/mL for 60 minutes at 37°C) abolishes the basal phosphorylation of Bad 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 Phospho-Bad (Ser112) Antibody #9296 (right panel) or Bad Antibody #9254 (left panel), are shown in the bottom figure.

U.S. Patent No. 5,675,063

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

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.

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Background References:

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(4) Peso, L. et al. (1997) *Science* 278, 687–689.

(5) Bonni, A. et al. (1999) *Science* 286, 1358–1362.

(6) Tan, Y. et al. (1999) *J. Biol. Chem.* 274, 34859–34867.

(7) Harada, H. et al. (1999) *Mol. Cell* 3, 413–422.

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(9) Lizcano, J. et al. (2000) *Biochem. J.* 349, 547–557.

(10) Datta, S. et al. (2000) *Mol. Cell* 6, 41–51.

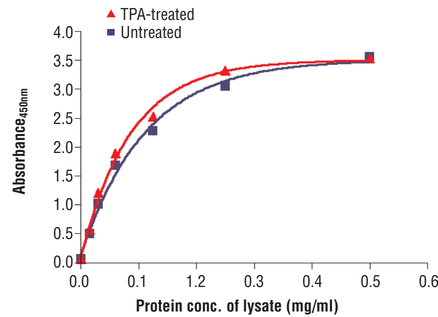

Figure 2. The relationship between the protein concentration of untreated and TPA-treated OVCAR8 cell lysates and the absorbance at 450 nm is shown. Cells were serum starved overnight and then treated with 200 nm TPA for 30 min. at 37°C.

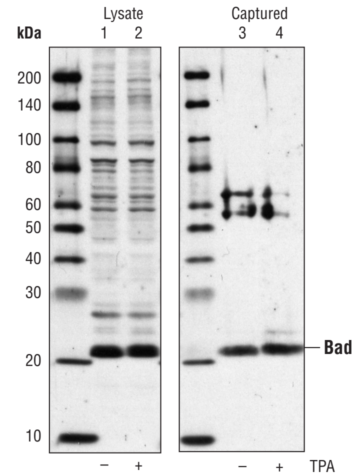

Figure 3. Kit specificity as demonstrated by Western analysis of the ELISA microwell captured protein. Lysates were prepared from OVCAR8 cells and incubated in microwells coated with the Bad capture antibody. Wells were washed, and the captured protein was solubilized in SDS gel loading buffer. Western analysis of OVCAR8 cell starting lysate (lanes 1 & 2) and the captured protein (lanes 3 & 4) was performed using Bad Mouse mAb #9254. The major band detected in the captured material corresponds to the Bad protein (lanes 3 & 4).

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PathScan® Sandwich ELISA Protocol (for kits with Lyophilized Antibodies)

A Solutions and Reagents

NOTE: Prepare solutions with purified water.

1. **Microwell strips:** Bring all to room temperature before use.
2. **Detection Antibody:** Supplied lyophilized as a green colored cake or powder. Add 1.0 ml of Detection Antibody Diluent (green solution) to yield a concentrated stock solution. Incubate at room temperature for 5 min with occasional gentle mixing to fully reconstitute. To make the final working solution, add the full 1.0 ml volume of reconstituted Detection Antibody to 10.0 ml of Detection Antibody Diluent in a clean tube and gently mix. Unused working solution may be stored for 4 weeks at 4°C.
3. **HRP-Linked Antibody*:** Supplied lyophilized as a red colored cake or powder Add 1.0 ml of HRP Diluent (red solution) to yield a concentrated stock solution. Incubate at room temperature for 5 min with occasional gentle mixing to fully reconstitute. To make the final working solution, add the full 1.0 ml volume of reconstituted HRP-Linked Antibody to 10.0 ml of HRP Diluent in a clean tube and gently mix. Unused working solution may be stored for 4 weeks at 4°C.
4. **Detection Antibody Diluent:** Green colored diluent for reconstitution and dilution of the detection antibody (11 ml provided).
5. **HRP Diluent:** Red colored diluent for reconstitution and dilution of the HRP-Linked Antibody (11 ml provided).
6. **Sample Diluent:** Blue colored diluent provided for dilution of cell lysates.
7. **1X Wash Buffer:** Prepare by diluting 20X Wash Buffer (included in each PathScan® Sandwich ELISA Kit) in purified water.
8. **Cell Lysis Buffer:** 10X Cell Lysis Buffer #9803: This buffer can be stored at 4°C for short-term use (1–2 weeks). Recommended: Add 1 mM phenylmethylsulfonyl fluoride (PMSF) immediately before use.
9. **TMB Substrate** (#7004).
10. **STOP Solution** (#7002).

**Note: Some PathScan® ELISA Kits may include HRP-Linked Streptavidin in place of HRP-Linked Antibody.*

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 (14,000 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 (~1200 rpm) when the culture reaches 0.5–1.0 x 10^6 viable cells/ml. Treat cells by adding fresh media containing regulator for desired time.
2. Collect cells by low speed centrifugation (~1200 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 (14,000 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 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 for each kit provide a sensitivity curve that serves as a reference for selection of an appropriate starting lysate concentration. The sensitivity curve shows typical kit assay results across a range of lysate concentration points.
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:
   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 reconstituted Detection Antibody (green color) to each well (refer to Section A, Step 2). Seal with tape and incubate the plate at 37°C for 1 hr.
6. Repeat wash procedure (Section C, Step 4).
7. Add 100 µl of reconstituted HRP-Linked secondary antibody (red color) to each well (refer to Section A, Step 3). Seal with tape and incubate the plate for 30 min at 37°C.
8. Repeat wash procedure (Section C, Step 4).
9. Add 100 µl 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 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 min after adding STOP Solution.
    b. Spectrophotometric Determination: Wipe underside of wells with a lint-free tissue. Read absorbance at 450 nm within 30 min after adding STOP Solution.

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