

PathScan® Phospho-Btk (Tyr223) Sandwich ELISA kit

#23843

1 Kit (96 assays)

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Entrez-Gene ID #695
UniProt ID #Q06187

rev. 03/18/20

For Research Use Only. Not For Use In Diagnostic Procedures.
Species Cross-Reactivity: H

Description: The PathScan® Phospho-Btk (Tyr223) Sandwich ELISA Kit is a solid phase sandwich enzyme-linked immunosorbent assay (ELISA) that detects endogenous levels of Btk protein phosphorylated at Tyr223. A Btk mouse antibody has been coated onto the microwells. After incubation with cell lysates, both phospho- and non-phospho-Btk proteins are captured by the coated antibody. Following extensive washing, a phospho-Btk (Tyr223) rabbit antibody is added to detect the captured phospho-Btk protein. Anti-rabbit IgG, HRP-linked Antibody 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 Btk phosphorylated at Tyr223.

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

Specificity/Sensitivity: PathScan® Phospho-Btk (Tyr223) Sandwich ELISA Kit detects endogenous levels of Btk protein phosphorylated at Tyr223 in human cells. The kit sensitivity is shown in Figure 1. This kit detects proteins from the indicated species, as determined through in-house testing, but may also detect homologous proteins from other species.

Background: Bruton's tyrosine kinase (Btk) is a member of the Btk/Tec family of cytoplasmic tyrosine kinases. Like other Btk family members, it contains a pleckstrin homology (PH) domain and Src homology SH3 and SH2 domains. Btk plays an important role in B cell development (1,2). Activation of B cells by various ligands is accompanied by Btk membrane translocation mediated by its PH domain binding to phosphatidylinositol-3,4,5-trisphosphate (3-5). The membrane-localized Btk is active and associated with transient phosphorylation of two tyrosine residues, Tyr551 and Tyr223. Tyr551 in the activation loop is transphosphorylated by the Src family tyrosine kinases, leading to autophosphorylation at Tyr223 within the SH3 domain, which is necessary for full activation (6,7). The activation of Btk is negatively regulated by PKC β through phosphorylation of Btk at Ser180, which results in reduced membrane recruitment, transphosphorylation, and subsequent activation (8). The PKC inhibitory signal is likely to be a key determinant of the B cell receptor signaling threshold to maintain optimal Btk activity (8).

Background References:

- (1) Khan, W.N. (2001) *Immunol Res* 23, 147-56.
- (2) Lewis, C.M. et al. (2001) *Curr Opin Immunol* 13, 317-25.
- (3) Salim, K. et al. (1996) *EMBO J* 15, 6241-50.
- (4) Rameh, L.E. et al. (1997) *J Biol Chem* 272, 22059-66.
- (5) Várnai, P. et al. (1999) *J Biol Chem* 274, 10983-9.
- (6) Rawlings, D.J. et al. (1996) *Science* 271, 822-5.
- (7) Park, H. et al. (1996) *Immunity* 4, 515-25.
- (8) Kang, S.W. et al. (2001) *EMBO J* 20, 5692-702.

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Applications: W—Western IP—Immunoprecipitation IHC—Immunohistochemistry ChIP—Chromatin Immunoprecipitation IF—Immunofluorescence F—Flow cytometry E-P—ELISA-Peptide **Species Cross-Reactivity:** 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.

| Products Includes | Item # | Kit Quantity | Color | Storage Temp |
|---|--------|--------------|---------------------|--------------|
| Btk Mouse mAb Coated Microwells* | 60976 | 96 tests | | 4°C |
| P-Btk (Tyr223) Rabbit Detection mAb | 88236 | 1 each | Green (Lyophilized) | 4°C |
| Anti-rabbit IgG, HRP-linked Antibody (ELISA Formulated) | 13272 | 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 each | | 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.

**Kit should be stored at 4°C with the exception of 10X Cell Lysis Buffer, which is stored at -20°C (packaged separately).

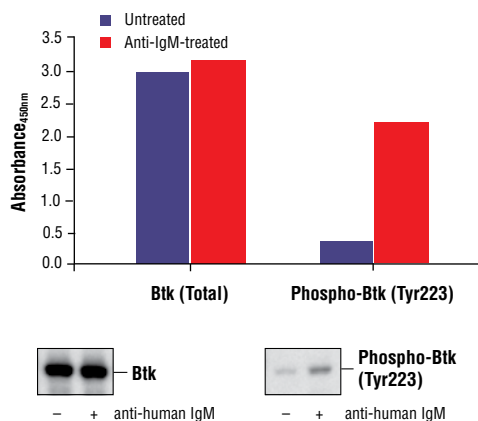


Figure 1. Treatment of Ramos cells with anti-human IgM stimulates phosphorylation of Btk at Tyr223, detected by PathScan® Phospho-Btk (Tyr223) Sandwich ELISA Kit #23843, but does not affect the level of total Btk, detected by PathScan® Total Btk Sandwich ELISA Kit #24547. The absorbance readings at 450 nm are shown in the top figure, while the corresponding western blots using Btk (D3H5) Rabbit mAb #8547 (left panel) or Phospho-Btk (Tyr223) (D9T6H) Rabbit mAb #87141 (right panel) are shown in the bottom figure.

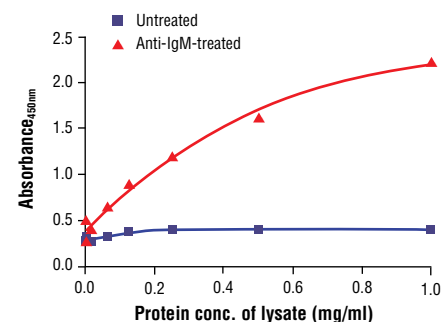


Figure 2. The relationship between the protein concentration of lysates from untreated and anti-human IgM-treated Ramos cells and the absorbance at 450 nm as detected by the PathScan® Phospho-Btk (Tyr223) Sandwich ELISA Kit is shown. Starved Ramos cells (1.0×10^6 cells/per ml) were treated with 12 μ g/ml of anti-human IgM for 10 min. at 37°C and then harvested and lysed.

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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 phenylmethyl-sulfonyl 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 × 10⁶ 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.