

#7324C Store at 4°C

PathScan® Phospho-ALK (Tyr1604) Sandwich ELISA Kit



1 Kit
 (96 assays)

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rev. 09/27/17

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

Species Cross-Reactivity: H

Description: CST's PathScan® Phospho-ALK (Tyr1604) Sandwich ELISA Kit is a solid phase sandwich enzyme-linked immunosorbent assay (ELISA) that detects endogenous levels of phospho-ALK (Tyr1604) or phospho-NPM-ALK fusion protein. A Phospho-ALK (Tyr1604) Antibody has been coated onto the microwells. After incubation with cell lysates, only phospho-ALK or phospho-NPM-ALK proteins are captured by the coated antibody. Following extensive washing, an ALK Mouse mAb is added to detect the captured phospho-ALK or phospho-NPM-ALK fusion 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 phospho-ALK (Tyr1604) or phospho-NPM-ALK proteins.

*Antibodies in kit are custom formulations specific to kit.

Specificity/Sensitivity: CST's PathScan® Phospho-ALK (Tyr1604) Sandwich ELISA Kit #7324 detects endogenous levels of phospho-ALK (Tyr1604) protein or phospho-NPM-ALK fusion protein. As shown in Figure 1, a high level of phosphorylated ALK (Tyr1604) protein or phospho-NPM-ALK fusion protein is detected in Karpas299 cells where ALK or NPM-ALK is constitutively phosphorylated. These high levels are abolished in Karpas299 cells lysed without addition of phosphatase inhibitors* to the lysis buffer. The levels of total ALK protein (phospho and nonphospho) detected by PathScan® Total ALK Sandwich ELISA Kit #7322 remain unchanged. This kit detects proteins from the indicated species, as determined through in-house testing, but may also detect homologous proteins from other species.

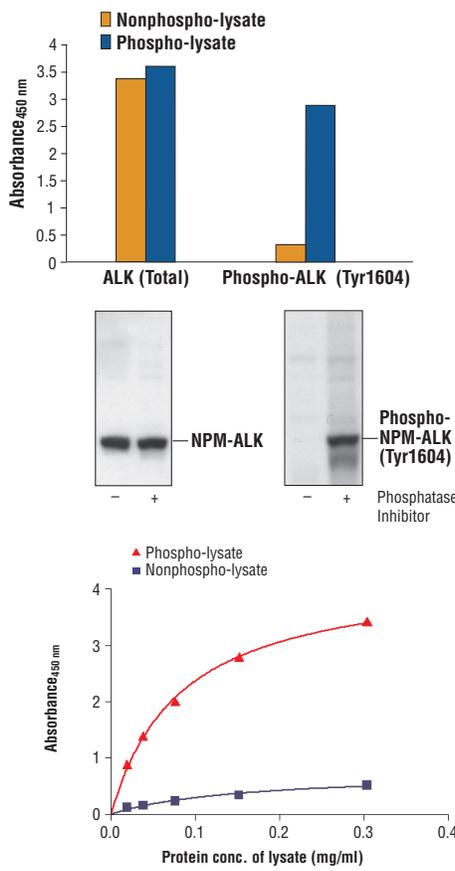
*Phosphatase inhibitors includes sodium pyrophosphate, β-glycerophosphate and Na₃VO₄.

Background: Anaplastic lymphoma kinase (ALK) is a tyrosine kinase receptor for pleiotrophin (PTN), a growth factor involved in embryonic brain development (1-3). In ALK-expressing cells, PTN induces phosphorylation of both ALK and the downstream effectors IRS-1, Shc, PLCγ, and PI3 kinase (1). ALK was originally discovered as an NPM (nucleophosmin)-ALK fusion protein produced by a translocation (4). The NPM-ALK fusion protein is a constitutively active, oncogenic tyrosine kinase associated with anaplastic lymphoma (4). Activation of PLCγ by NPM-ALK has been suggested to be a crucial step for its mitogenic activity and may be important in the pathogenesis of anaplastic lymphomas (5). A distinct ALK oncogenic fusion protein involving ALK and EML4 has been described from a non-small cell lung cancer cell line, with corresponding fusion transcripts present in some cases of lung adenocarcinoma. The short, amino-terminal region of the microtubule-associated protein EML4 is fused to the kinase domain of ALK (6,7).

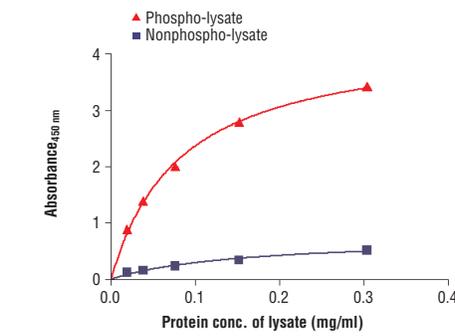
Applications Key: W—Western IP—Immunoprecipitation IHC—Immunohistochemistry IC—Immunocytochemistry IF—Immunofluorescence F—Flow cytometry E—ELISA D—DELFI[®]
Species Cross-Reactivity Key: H—human M—mouse R—rat Hm—hamster Mk—monkey Mi—mink C—chicken X—Xenopus Z—zebra fish B—bovine All—all species expected
 Species enclosed in parentheses are predicted to react based on 100% sequence homology.

Product Includes	Item #	Kit Quantity	Color	Storage Temp
Phospho-ALK (Y1604) Rabbit Antibody Coated Microwells*	88910	96 tests		4°C
ALK Mouse Detection mAb	13993	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:** Constitutive phosphorylation of NPM-ALK in Karpas299 cells lysed in the presence of phosphatase inhibitors (phospho-lysate) can be detected by PathScan® Phospho-ALK (Tyr1604) Sandwich ELISA Kit #7324. In contrast, only a low level of phospho-NPM-ALK protein is detected in Karpas299 cells lysed without addition of phosphatase inhibitors to the lysis buffer (nonphospho-lysate). However, similar levels of total NPM-ALK protein from either nonphospho or phospho-lysates can be detected by PathScan® Total ALK Sandwich ELISA Kit #7322. Absorbance at 450 nm is shown in the top figure, while the corresponding Western blots using Phospho-ALK (Tyr1604) Antibody #3341 (right panel) or a total ALK Rabbit mAb #3333 (left panel), are shown in the bottom figure.



◀ **Figure 2:** The relationship between protein concentration of phospho or non-phospho lysates and the absorbance at 450 nm is shown. Unstarved Karpas299 cells were cultured (10⁶ cells/ml) and lysed with or without addition of phosphatase inhibitor to the lysis buffer (phospho or non-phospho lysate).

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

A Solutions and Reagents

NOTE: Prepare solutions with purified water.

- Microwell strips:** Bring all to room temperature before use.
- 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.
- 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.
- Detection Antibody Diluent:** Green colored diluent for reconstitution and dilution of the detection antibody (11 ml provided).
- HRP Diluent:** Red colored diluent for reconstitution and dilution of the HRP-Linked Antibody (11 ml provided).
- Sample Diluent:** Blue colored diluent provided for dilution of cell lysates.
- 1X Wash Buffer:** Prepare by diluting 20X Wash Buffer (included in each PathScan® Sandwich ELISA Kit) in purified water.
- 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.
- TMB Substrate** (#7004).
- 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.

- Aspirate media when the culture reaches 80–90% confluence. Treat cells by adding fresh media containing regulator for desired time.
- Remove media and rinse cells once with ice-cold 1X PBS.
- 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.
- Scrape cells off the plate and transfer to an appropriate tube. Keep on ice.
- Sonicate lysates on ice.
- 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

- 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.
- Collect cells by low speed centrifugation (~1200 rpm) and wash once with 5–10 ml ice-cold 1X PBS.
- Cells harvested from 50 ml of growth media can be lysed in 2.0 ml of 1X Cell Lysis Buffer plus 1 mM PMSF.
- Sonicate lysates on ice.
- 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

- 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.
- 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.
- 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.
- Gently remove the tape and wash wells:
 - Discard plate contents into a receptacle.
 - Wash 4 times with 1X Wash Buffer, 200 µl each time for each well.
 - 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.
 - Clean the underside of all wells with a lint-free tissue.
- 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.
- Repeat wash procedure (Section C, Step 4).
- 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.
- Repeat wash procedure (Section C, Step 4).
- 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.
- 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.

- Read results.
 - Visual Determination:** Read within 30 min after adding STOP Solution.
 - Spectrophotometric Determination:** Wipe underside of wells with a lint-free tissue. Read absorbance at 450 nm within 30 min after adding STOP Solution.

Background References:

- (1) Stoica, G.E. et al. (2001) *J Biol Chem* 276, 16772–9.
- (2) Iwahara, T. et al. (1997) *Oncogene* 14, 439–49.
- (3) Morris, S.W. et al. (1997) *Oncogene* 14, 2175–88.
- (4) Morris, S.W. et al. (1994) *Science* 263, 1281–4.
- (5) Bai, R.Y. et al. (1998) *Mol Cell Biol* 18, 6951–61.
- (6) Rikova, K. et al. (2007) *Cell* 131, 1190–203.
- (7) Takeuchi, K. et al. (2008) *Clin Cancer Res* 14, 6618–24.