

# PathScan® Total ALK Chemiluminescent Sandwich ELISA Kit



Cell Signaling  
TECHNOLOGY®

✓ 1 Kit  
(96 assays)

Low volume microplate

Orders ■ 877-616-CELL (2355)  
orders@cellsignal.com

Support ■ 877-678-TECH (8324)  
info@cellsignal.com

Web ■ www.cellsignal.com

New 10/09

This product is for *in vitro* research use only and is not intended for use in humans or animals.  
This product is not intended for use as a therapeutic or in diagnostic procedures.

Entrez-Gene ID #238  
Swiss-Prot Acc. #Q9UM73

## Species Cross-Reactivity: H

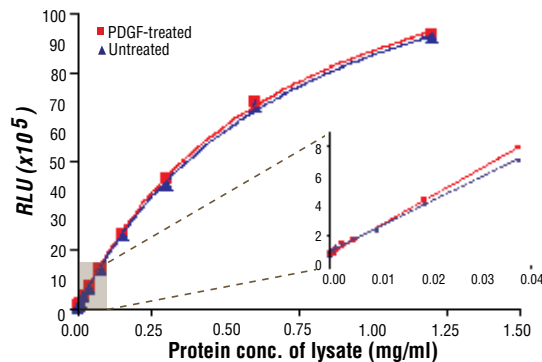
**Description:** The PathScan® Total ALK Chemiluminescent Sandwich ELISA Kit is a solid phase sandwich enzyme-linked immunosorbent assay (ELISA) that detects endogenous levels of total ALK protein and EML4-ALK or NPM-ALK fusion proteins with a chemiluminescent readout. Chemiluminescence ELISAs often have a wider dynamic range and higher sensitivity than conventional chromogenic detection. This chemiluminescent ELISA, which is offered in low volume microplates, shows increased signal and sensitivity while using a smaller sample size. An ALK rabbit antibody has been coated onto the microwells. After incubation with cell lysates, ALK and ALK fusion proteins are captured by the coated antibody. Following extensive washing, an ALK mouse antibody is added to detect the captured ALK and ALK fusion proteins. Anti-mouse IgG, HRP-linked antibody is then used to recognize the bound detection antibody. Chemiluminescent reagent is added for signal development. The magnitude of light emission, measured in relative light units (RLU) is proportional to the quantity of total ALK and ALK fusion proteins.

**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, PLC $\gamma$  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 PLC $\gamma$  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).

**Specificity/Sensitivity:** PathScan® Total ALK Chemiluminescent Sandwich ELISA Kit #7084 detects endogenous levels of total ALK, EML4-ALK and NPM-ALK fusion proteins in human cells.

Products Included	Volume	Solution Color
ALK Rabbit Antibody Coated Microwells*	96 tests	
ALK Mouse Detection mAb	5.5 ml	Green
Anti-mouse IgG, HRP-linked Antibody	5.5 ml	Red
Luminol/Enhancer Solution	3 ml	Colorless
Stable Peroxide Buffer	3 ml	Colorless
Sealing Tape	2 sheets	
20X Wash Buffer	25 ml	Colorless
Sample Diluent	25 ml	Blue
**Cell Lysis Buffer #9803	15 ml	Yellowish

**Low volume microplate** \* 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).



Relationship between protein concentrations of lysates prepared using NCI-H2228 cells lysed with (phospho) and without (nonphospho) the addition of phosphatase inhibitors to the lysis buffer. Graph inset corresponding to the shaded area shows high sensitivity and a linear response at the low protein concentration range.

## 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.

## Chemiluminescent ELISA Protocol

**NOTE:** This chemiluminescent ELISA is offered in low volume microplate. Samples and reagents only require 50 µl per microwell.

### A Required Reagents

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: (10X Cell Lysis Buffer #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<sub>3</sub>VO<sub>4</sub>, 1 µg/ml leupeptin. This buffer can be stored at 4°C for short-term use (1–2 weeks).  
**Note:** CST recommends adding 1 mM PMSF immediately before use.

### 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 50 µl of Sample Diluent (supplied in each Pathscan® Sandwich ELISA Kit, blue color) to a microcentrifuge tube. Transfer 50 µl of cell lysate into the tube and vortex for a few seconds. (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.
3. Add 50 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 room temperature (RT). 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, 150 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 dry completely at any time.
  - d. Clean the underside of all wells with a lint-free tissue.
5. Add 50 µl of Detection Antibody (green color) to each well. Seal with tape and incubate the plate for 1 hour at room temperature.
6. Repeat wash procedure as in Step C4.
7. Add 50 µl of HRP-linked secondary antibody (red color) to each well. Seal with tape and incubate the plate for 30 minutes at room temperature.
8. Repeat wash procedure as in Step C4.
9. Prepare Working Solution by mixing equal parts Luminol/Enhancer Solution and Stable Peroxide Buffer.
10. Add 50 µl of the Working Solution to each well.

Use a plate-based luminometer to measure Relative Light Units (RLU) at 425nM within 1-10 minutes following addition of the substrate.

Optimal signal intensity is achieved when read within 10 minutes.