



#7084 Store at +4C

PathScan® Total ALK Chemiluminescent Sandwich ELISA Kit

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1 Kit (96 assays)

Species Cross Reactivity: H
UniProt ID: #Q9UM73
Entrez-Gene Id: #238

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For Research Use Only. Not for Use in Diagnostic Procedures.

| Product Includes | Product # | Quantity | Color | Storage Temp |
|--|-----------|----------|---------------------|--------------|
| ALK Rabbit mAb Coated Microwells | 19900 | 96 tests | | +4C |
| ALK Mouse Detection mAb | 13993 | 1 ea | Green (Lyophilized) | +4C |
| Anti-mouse IgG, HRP-linked Antibody (ELISA Formulated) | 13304 | 1 ea | Red (Lyophilized) | +4C |
| Detection Antibody Diluent | 13339 | 5.5 ml | Green | +4C |
| HRP Diluent | 13515 | 5.5 ml | Red | +4C |
| Luminol/Enhancer Solution | 84850 | 3 ml | | RT |
| Stable Peroxide Buffer | 42552 | 3 ml | | RT |
| Sealing Tape | 54503 | 2 ea | | +4C |
| ELISA Wash Buffer (20X) | 9801 | 25 ml | | +4C |
| ELISA Sample Diluent | 11083 | 25 ml | Blue | +4C |
| Cell Lysis Buffer (10X) | 9803 | 15 ml | | -20C |

Kit contents scale proportionally with size, except sealing tape.

Example: The V1 kit contains 5X the listed quantities above, but will exclude the sealing tape.

The microwell plate is supplied as 12 8-well modules - Each module is designed to break apart for 8 tests.

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 magnitude of light emission, measured in relative light units (RLU) is proportional to the quantity of total ALK and ALK fusion proteins.

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

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. This kit detects proteins from the indicated species, as determined through in-house testing, but may also detect homologous proteins from other species.

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 a nucleophosmin (NPM)-ALK fusion protein produced by a translocation (4). Investigators have found that the NPM-ALK fusion protein is a constitutively active, oncogenic tyrosine kinase associated with anaplastic lymphoma (4). Research literature suggests that activation of PLCγ by NPM-ALK may be a crucial step for its mitogenic activity and involved in the pathogenesis of anaplastic lymphomas (5).

A distinct ALK oncogenic fusion protein involving ALK and echinoderm microtubule-associated protein like 4 (EML4) has been described in the research literature from a non-small cell lung cancer (NSCLC) 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 (6-8).

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.
 8. Soda, M. et al. (2007) *Nature* 448, 561-6.
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#7084

PathScan® Total ALK Chemiluminescent Sandwich ELISA Kit



ELISA Chemiluminescent (Lyophilized)

NOTE: Refer to product-specific datasheets for assay incubation temperature. This chemiluminescent ELISA is offered in low volume microplates. Only 50 µl of samples or reagents are required in each microwell.

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 0.5 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 0.5 ml volume of reconstituted Detection Antibody to 5.0 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 0.5 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 0.5 ml volume of reconstituted HRP-Linked Antibody to 5.0 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.
5. **HRP Diluent:** Red colored diluent for reconstitution and dilution of the HRP-Linked Antibody.
6. **Sample Diluent:** Blue colored diluent 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:** PathScan® Sandwich ELISA Lysis Buffer (1X) #7018: 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. **Luminol/Enhancer Solution and Stable Peroxide Buffer.**

***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 to 1 ml ice-cold PathScan® Sandwich ELISA Lysis Buffer (1X) #7018 plus 1 mM PMSF to each plate (10 cm diameter) and incubate the plate on ice for 2 min.
4. Collect cell lysate in a clean tube.
5. Centrifuge for 10 min (14,000 x g) at 4°C and transfer the supernatant to a new tube. Store supernatant 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 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. Resuspend the cell pellet and incubate the tube for 2 min.
5. Microcentrifuge for 10 min (x14,000 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 50 μ l 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 room temperature. Alternatively, the plate can be incubated overnight at 4°C.
4. Gently remove the tape and wash wells:
 1. Discard plate contents into a receptacle.
 2. Wash 4 times with 1X Wash Buffer, 150 μ l each time for each well.
 3. 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.
 4. Clean the underside of all wells with a lint-free tissue.
5. Add 50 μ l of reconstituted Detection Antibody (green color) to each well (refer to Section A, Step 2). Seal with tape and incubate the plate at room temperature for 1 hr.
6. Repeat wash procedure (Section C, Step 4).
7. Add 50 μ l of reconstituted HRP-linked secondary antibody (red color) to each well (refer to Section A, Step 3). Seal with tape and incubate the plate at room temperature for 30 min.
8. Repeat wash procedure (Section C, Step 4).
9. Prepare Detection Reagent Working Solution by mixing equal parts Luminol/Enhancer Solution and Stable Peroxide Buffer.
10. Add 50 μ l of the Detection Reagent Working Solution to each well.
11. Use a plate-based luminometer to measure Relative Light Units (RLU) at 425 nm within 1–10 min following addition of the substrate. Optimal signal intensity is achieved when read within 10 min.

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