

PathScan® Total Axl Sandwich ELISA Kit

✓ 1 Kit
(96 assays)



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This product is intended for research purposes only. This product is not intended to be used for therapeutic or diagnostic purposes in humans or animals.

Entrez-Gene ID #558
Swiss-Prot Acc. #P30530

Species Cross-Reactivity: H

Description: The PathScan® Total Axl Sandwich ELISA Kit is a solid phase sandwich enzyme-linked immunosorbent assay (ELISA) that detects endogenous levels of total Axl protein. An Axl mouse antibody has been coated on the microwells. After incubation with cell lysates, Axl protein (phospho and nonphospho) is captured by the coated antibody. Following extensive washing, an Axl rabbit antibody is added to detect captured Axl 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 total Axl protein.

Specificity/Sensitivity: CST's PathScan® Total Axl Sandwich ELISA Kit #7040 detects endogenous levels of total Axl protein in human cells, as shown in Figure 1. The kit sensitivity is shown in Figure 2.

Background: Axl, Sky and Mer are three members of a receptor tyrosine kinase (RTK) family that share a conserved intracellular tyrosine kinase domain and an extracellular domain similar to those seen in cell adhesion molecules. These RTKs bind the vitamin K-dependent protein growth-arrest-specific 6 (Gas6), which is structurally related to the protein S anticoagulation factor (1). Upon binding to its receptor, Gas6 activates phosphatidylinositol 3-kinase (PI3K) and its downstream targets Akt and S6K, as well as NF-κB (2,3). A large body of evidence supports a role for Gas6/Axl signaling in cell growth and survival in normal and cancer cells (4).

Background References:

- (1) Crosier, K.E. and Crosier, P.S. (1997) *Pathology* 29, 131-135.
- (2) Demarchi, F. et al. (2001) *J. Biol. Chem.* 276, 31738-31744.
- (3) Lee, W. P. et al. (2002) *Oncogene* 21, 329-336.
- (4) Bellosta, P. et al. (1997) *Oncogene* 15, 2387-2397.

Products Included	Volume	Solution Color
Axl Mouse Antibody Coated Microwells*	96 tests	
Axl Rabbit Detection Antibody	11 ml	Green
Anti-rabbit IgG, HRP-linked Antibody	11 ml	Red
TMB Substrate	11 ml	Colorless
STOP Solution	11 ml	Colorless
Sealing Tape	2 sheets	
20X Wash Buffer	25 ml	Colorless
Sample Diluent	25 ml	Blue
10X Cell Lysis Buffer #9803**	15 ml	Yellowish

* 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).

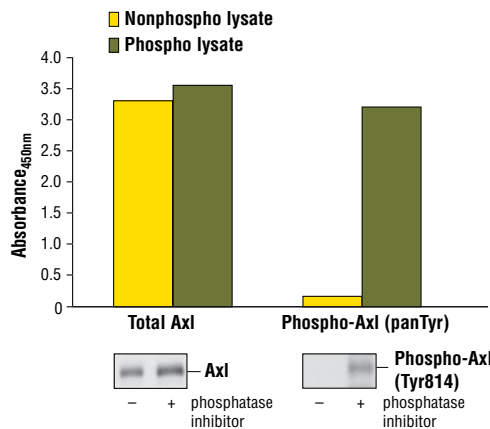


Figure 1: Constitutive phosphorylation of Axl in NCI-H2347 cells lysed in the presence of phosphatase inhibitors* (phospho lysate) is detected by PathScan® Phospho-Axl (panTyr) Sandwich ELISA Kit #7042 (top, right). In contrast, a low level of phospho-Axl protein is detected in NCI-H2347 cells lysed in the absence of phosphatase inhibitors* (nonphospho lysate). Similar levels of total Axl protein from both nonphospho or phospho lysates are detected by PathScan® Total Axl Sandwich ELISA Kit #7040 (top, left). Absorbance at 450 nm is shown in the top figure while corresponding western blots using a phospho-Axl (Tyr814) rabbit antibody (right) or a total Axl (C44G1) Rabbit mAb #4566 (left) are shown in the bottom figure. *Phosphatase inhibitors include sodium pyrophosphate, β-glycerophosphate and Na₃VO₄.

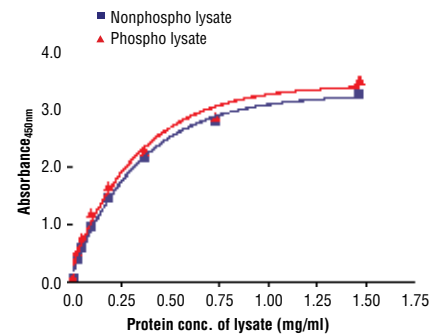


Figure 2: The relationship between protein concentration of phospho or nonphospho lysates and the absorbance at 450 nm is shown. NCI-H2347 cells were cultured (85% confluence) and lysed with or without the addition of phosphatase inhibitor to the lysis buffer (phospho or nonphospho lysate).

Sandwich ELISA Protocol

A Reagent Preparation

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 from CST #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_2VO_4 , 1 $\mu\text{g}/\text{ml}$ leupeptin. This buffer can be stored at 4°C for short-term use (1–2 weeks).

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 100 μl of Sample Diluent (supplied in each PathScan® Sandwich ELISA Kit, blue color) to a microcentrifuge tube. Transfer 100 μl of cell lysate into the tube and vortex for a few seconds. Generally, 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. However, dilution factors need to be titrated when specific cell lysates are used.

3. Add 100 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 37°C. 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, 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 Detection Antibody (green color) to each well. Seal with tape and incubate the plate for 1 hour at 37°C.
6. Repeat wash procedure as in Step 4.
7. Add 100 μl of HRP-linked secondary antibody (red color) to each well. Seal with tape and incubate the plate for 30 minutes at 37°C.
8. Repeat wash procedure as in Step 4.
9. Add 100 μl of TMB Substrate to each well. Seal with tape and incubate the plate for 10 minutes at 37°C or 30 minutes 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 minutes after adding STOP Solution.
 - b. Spectrophotometric Determination — Wipe underside of wells with a lint-free tissue. Read absorbance at 450 nm within 30 minutes after adding STOP Solution.