

# PathScan® Phospho-LAT (Tyr191) Sandwich ELISA Kit

✓ 1 Kit  
(96 assays)

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

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

**Web** ■ www.cellsignal.com

rev. 09/06/12

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

Entrez-Gene ID #4233  
Swiss-Prot Acc. #P08581

## Species Cross-Reactivity: H

**Introduction:** The PathScan® Phospho-LAT (Tyr191) Sandwich ELISA Kit is a solid phase sandwich enzyme-linked immunosorbent assay (ELISA) that detects endogenous levels of phospho-LAT (Tyr191). A LAT mouse antibody has been coated onto the microwells. After incubation with cell lysates, LAT protein (phosphorylated and non-phosphorylated) is captured by the coated antibody. Following extensive washing, a phospho-LAT (Tyr191) rabbit detection antibody is added to detect the captured phospho-LAT (Tyr191). HRP-linked anti-rabbit antibody is then used to recognize the bound detection antibody. The HRP substrate TMB is added to develop color. The magnitude of the absorbance for this developed color is proportional to the quantity of phospho-LAT (Tyr191).

Please visit [www.cellsignal.com](http://www.cellsignal.com) for a complete listing of recommended companion products.

**Specificity/Sensitivity:** CST's PathScan® Phospho-LAT (Tyr191) Sandwich ELISA Kit detects endogenous levels of Phospho-LAT when phosphorylated at Tyr191. As shown in Figure 1, a significant induction of LAT phosphorylation at Tyr191 can be detected in Jurkat cells following treatment with anti-CD3 using the Phospho-LAT (Tyr191) Sandwich ELISA Kit. The level of total LAT remains unchanged as shown by western analysis (Figure 1).

**Background:** LAT, a transmembrane adaptor protein expressed in T, NK and mast cells, is an important mediator for T cell receptor (TCR) signaling (1). Upon TCR engagement, activated Zap-70 phosphorylates LAT at multiple conserved tyrosine residues within SH2 binding motifs, exposing these motifs as the docking sites for downstream signaling targets (2,3). The phosphorylation of LAT at Tyr171 and Tyr191 enables the binding of Grb2, Gads/SLP-76, PLC $\gamma$ 1 and PI3 kinase through their SH2 domain and translocates them to the membrane. This process eventually leads to activation of the corresponding signaling pathways (1-4).

## Background References:

- (1) Wonerow, P. and Watson, S.P. (2001) *Oncogene* 20, 6273-6283.
- (2) Zhang, W. et al. (1998) *Cell* 92, 83-92.
- (3) Paz, P. E. et al. (2001) *Biochem. J.* 356, 461-471.
- (4) Zhang, W. et al. (2000) *J. Biol. Chem.* 275, 23355-23361.

Products Included	Volume	Solution Color
LAT Mouse Antibody Coated Microwells*	96 tests	
Phospho-LAT (Tyr191) 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).

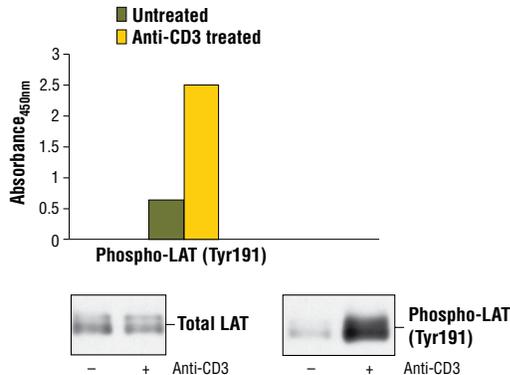


Figure 1. Treatment of Jurkat cells with anti-CD3 stimulates phosphorylation of LAT at Tyr191 as detected by the PathScan® Phospho-LAT (Tyr191) Sandwich ELISA Kit #7936, but does not affect the level of total LAT. Jurkat cells were starved for 48 hours and treated with 10  $\mu$ g/ml anti-CD3 for 2 minutes at 37°C. The absorbance readings at 450 nm are shown in the top figure, while the western blots, using LAT Antibody #9166 (left panel) or Phospho-LAT (Tyr191) Antibody #3584 (right panel), are shown in the bottom figure.

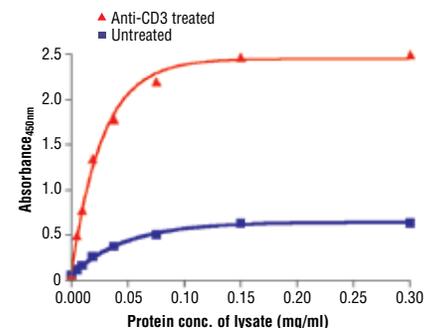


Figure 2. The relationship between lysate protein concentration from untreated and anti-CD3-treated Jurkat cells and the absorbance at 450 nm is shown.

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

**Species Cross-Reactivity Key:** 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 All—all species expected Species enclosed in parentheses are predicted to react based on 100% sequence homology.

## 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  $\beta$ -glycerophosphate, 1 mM  $\text{Na}_3\text{VO}_4$ , 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  $\mu\text{l}$  of Sample Diluent (supplied in each PathScan® Sandwich ELISA Kit, blue color) to a microcentrifuge tube. Transfer 100  $\mu\text{l}$  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  $\mu\text{l}$  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  $\mu\text{l}$  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  $\mu\text{l}$  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  $\mu\text{l}$  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  $\mu\text{l}$  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  $\mu\text{l}$  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.