Store at 4°C and –20°C

#7937

PathScan® Phospho-LAT (Tyr191) Sandwich ELISA Antibody Pair

✓ 1 Kit
(4 X 96 well plates)

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New 03/09

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 #6850
Swiss-Prot Acc. #P43405

Species Cross-Reactivity: H

Introduction: CST's PathScan® Phospho-LAT (Tyr191) Sandwich ELISA Antibody Pair is being offered as an economical alternative to our PathScan® Phospho-LAT (Tyr191) Sandwich ELISA Kit #7936. Capture and detection antibodies (100X stocks) and an HRP-conjugated secondary antibody (1000X stock) are supplied. Sufficient reagents are supplied for 4 x 96 well ELISAs. The LAT mouse capture antibody is coated onto a 96 well microplate overnight in PBS. After blocking, cell lysate is added followed by a phospho-LAT (Tyr191) rabbit detection antibody and HRP-conjugated, anti-rabbit IgG antibody. HRP substrate (TMB) is then added for color development. The magnitude of the absorbance for this developed color is proportional to the quantity of phospho-LAT (Tyr191).

Reagents not supplied:

Phosphate Buffered Saline (PBS-20X) #9808

Phosphate Buffered Saline with Tween -20 (PBST-20X) #9809

Cell Lysis Buffer (10X) #9803

TMB Substrate #7004

STOP Solution #7002

Blocking Buffer- PBS+0.05% Tween-20, 1% BSA

96 Well Microplates**

Microplate Reader

** Antibody Pairs have been validated on Corning® 96 Well Clear Polystyrene High Bind Stripwell™ Microplates (#2592) and Corning® 96 Well EIA/RIA Easy Wash™ Clear Flat Bottom Polystyrene High Bind Microplates (#3369).

Note: Antibody pairs have been optimized using recommended buffers, reagents, plates and protocol. Solutions should be made fresh daily.

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 191 enables the binding of Grb2, Gads/SLP-76, PLCγ1 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).

Products Included	Volume	Cap Color	Storage
LAT Mouse Capture Antibody (100X)	0.4 ml	Pink	4°C
Phospho-LAT (Tyr191) Rabbit Detection Antibody (100X)	0.4 ml	Blue	4°C
Anti-rabbit IgG, HRP-linked Antibody (1000X)	0.04 ml	Yellow	–20°C

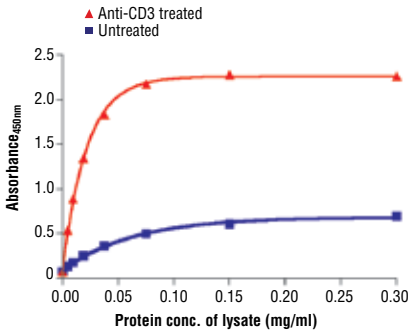

Figure 1. The relationship between the protein concentration of the lysate from untreated and anti-CD3-treated Jurkat cells and the absorbance at 450 nm using the PathScan® Phospho-LAT (Tyr191) Sandwich ELISA Antibody Pair #7937 is shown. After a 48 hour starvation, Jurkat cells were treated with anti-CD3 (10 µg/ml) for 2 minutes at 37°C and then lysed.

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.

Storage: LAT-specific mouse capture and phospho-LAT (Tyr191)-specific rabbit detection antibodies are stored at 4°C. HRP-linked, anti-rabbit IgG antibody is stored at -20°C.

Companion Products:

LAT Antibody #9166

Phospho-LAT (Tyr191) Antibody #3584

Anti-rabbit IgG, HRP-linked Antibody #7074

Cell Lysis Buffer (10X) #9803

TMB Substrate #7004

STOP Solution #7002

BSA #9998

Phosphate Buffered Saline (PBS-20X) #9808

Phosphate Buffered Saline with Tween 20 (PBST-20X) #9809

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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**—zebra fish **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.

#7937

PathScan® Sandwich ELISA Antibody Pair Protocol

A Required Reagents

1. **Coating Buffer:** 1X PBS, (20X PBS #9808)
   3.2 mM Na_2HPO_4, 0.5 mM KH_2PO_4, 1.3 mM KCl, 135 mM NaCl, pH 7.4
2. **Wash Buffer:** 1X PBS/0.05% Tween-20, (20X PBST #9809)
3. **Blocking Buffer:** 1X PBS/0.05% Tween-20, 1% BSA
4. **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_3VO_4, 1 µg/ml leupeptin.
5. **TMB Substrate:** (TMB Substrate #7004)
6. **STOP Solution:** (STOP Solution #7002)

NOTE: Reagents should be made fresh daily

B Coating Procedure

1. Rinse microplate with dH_2O. Add 200 µl of dH_2O and discard liquid. Blot on paper towel to make sure wells are dry.
2. Dilute capture antibody 1:100 in PBS. For a single 96 well plate, add 100 µl of Capture Antibody Stock to 9.9 ml PBS. Mix well and add 100 µl/well. Cover plate and incubate overnight at 4°C (17-20 hours).
3. **After overnight coating, gently uncover plate and wash wells:**
   a. Discard plate contents into a receptacle.
   b. Wash 4 times with 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.
   c. Clean the underside of all wells with a lint-free tissue.
4. Block plates. Add 150 µl of Blocking Buffer/well, cover plate and incubate at 37°C for 2 hours.
5. After blocking, wash plate as in Step 3. Plate is ready to use.

C 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 phenylmethylsulfonyl fluoride (PMSF) to each plate (10 cm diameter plate) 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.

D Test Procedure

1. Lysates can be used undiluted or diluted in Blocking Buffer. 100 µl of lysate is added per well. Cover plate and incubate at 37°C for 2 hours.
2. Wash plate as in Coating Procedure, Step 3.
3. Dilute detection antibody 1:100 in Blocking Buffer. For a single 96 well plate, add 100 µl of Detector Antibody Stock to 9.9 ml of Blocking Buffer. Mix well and add 100 µl/well. Cover plate and incubate at 37°C for 1 hour.
4. Plate is washed as in Coating Procedure, Step 3.
5. Secondary antibody, either, anti-mouse or anti-rabbit-HRP, is diluted 1:1000 in Blocking Buffer. For a single 96 well plate, add 10 µl of secondary antibody stock to 9.99 ml of Blocking Buffer. Mix well and add 100 µl/well. Cover and incubate at 37°C for 30 minutes.
6. Wash plate as in Coating Procedure, Step 3.
7. Add 100 µl of TMB Substrate per well. Cover and incubate at 37°C for 10 minutes.
8. Add 100 µl of STOP Solution per well.
9. Read plate on a microplate reader at Absorbance 450 nm.

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