

T Cell Signaling Antibody Sampler Kit

✓ 1 Kit
(7 x 40 µl)

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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.

Products Included	Product #	Quantity	Mol. Wt.	Isotype
Zap-70 (D1C10E) XP™ Rabbit mAb	3165	40 µl	70 kDa	Rabbit IgG
Phospho-Zap-70 (Tyr319)/Syk (Tyr352) (65E4) Rabbit mAb	2717	40 µl	70, 72 kDa	Rabbit IgG
SLP-76 Antibody	4958	40 µl	76 kDa	Rabbit IgG
Phospho-LAT (Tyr191) Antibody	3584	40 µl	36, 38 kDa	Rabbit IgG
LAT Antibody	9166	40 µl	36, 38 kDa	Rabbit IgG
Phospho-Lck (Tyr505) Antibody	2751	40 µl	56 kDa	Rabbit IgG
Lck (73A5) Rabbit mAb	2787	40 µl	56 kDa	Rabbit IgG
Anti-rabbit IgG, HRP-linked Antibody	7074	100 µl		Goat

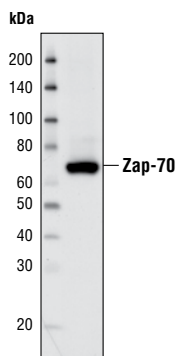
See www.cellsignal.com for individual component applications, species cross-reactivity, dilutions and additional application protocols.

Description: The T Cell Signaling Antibody Sampler Kit contains primary and secondary antibodies to perform four Western mini-blot with each antibody.

Background: Zap-70, a Syk family protein tyrosine kinase expressed in T and NK cells, plays a critical role in mediating T cell activation in response to T cell receptor (TCR) engagement (1). Following TCR engagement, Zap-70 is rapidly phosphorylated on several tyrosine residues, presumably by two mechanisms: autophosphorylation and transphosphorylation by the Src family tyrosine kinase, Lck (2-6). Tyrosine phosphorylation of Zap-70 correlates with its increased kinase activity and downstream signaling events. In patients with chronic lymphocytic leukemia (CLL), total Zap-70 expression was shown to be correlated with disease progression and survival (7,8).

LAT, a transmembrane adaptor protein expressed in T, NK and mast cells, is an important mediator for T cell receptor (TCR) signaling (9). 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 (10,11). The phosphorylation of LAT at Tyr171 and 191 enables the binding of Grb2, Gads/SLP-76, PLCgamma1 and PI3 kinase through their SH2 domain and translocates them to the membrane. This process eventually leads to activation of the corresponding signaling pathways (11-12).

Specificity/Sensitivity: Each antibody in the T Cell Signaling Antibody Sampler Kit recognizes only its specific target and does not cross-react with other family members with the following exceptions: Phospho-Zap-70 (Tyr319)/Syk (Tyr352) (65E4) Rabbit mAb which also recognizes Syk when phosphorylated at Tyr352; and Phospho-Lck (Tyr505) Antibody which may cross-react with certain phosphorylated Src family members.



Western blot analysis of extracts from Jurkat cells using Zap-70 (D1C10E) XP™ Rabbit mAb #3165.

Source/Purification: Monoclonal antibodies are produced by immunizing animals with a synthetic peptide (KLH-coupled) corresponding to residues surrounding the amino terminus of human Zap-70, Tyr319 of human Zap-70 and Tyr505 of human Lck.

Polyclonal antibodies are produced by immunizing animals with a synthetic peptide (KLH-coupled) corresponding to the sequence of human SLP-76; residues surrounding Tyr191 of human LAT; the central region of human LAT; residues surrounding Tyr505 of human Lck. Antibodies are purified by protein A and peptide affinity chromatography.

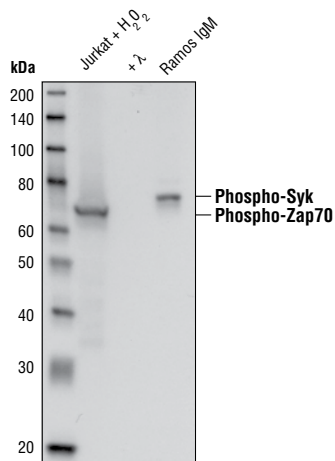
Storage: Supplied in 10 mM sodium HEPES (pH 7.5), 150 mM NaCl, 100 µg/ml BSA and 50% glycerol. Store at -20°C. Do not aliquot the antibodies.

Recommended Antibody Dilutions:
Western blotting 1:1000

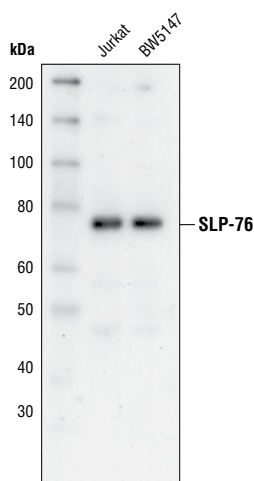
Please visit www.cellsignal.com for a complete listing of recommended companion products.

Background References:

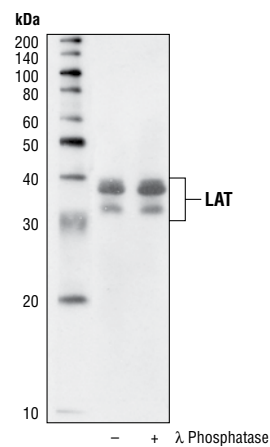
- (1) Chu, D.H. et al. (1998) *Immunol. Rev.* 165, 167–180.
- (2) Iwashima, M. et al. (1994) *Science* 263, 1136–1139.
- (3) Neumeister, E.N. et al. (1995) *Mol. Cell Biol.* 15, 3171–3178.
- (4) Chan, A.C. et al. (1995) *EMBO J.* 14, 2499–2508.
- (5) Williams, B.L. et al. (1999) *EMBO J.* 18, 1832–1844.
- (6) Di Bartolo, V. et al. (1999) *J. Biol. Chem.* 274, 6285–6294.
- (7) Wiestner, A. et al. (2003) *Blood* 101, 4944–4951.
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- (9) Wonerow, P. and Watson, S.P. (2001) *Oncogene* 20, 6273–83.
- (10) Zhang, W. et al. (1998) *Cell* 92, 83–92.
- (11) Paz, P.E. et al. (2001) *Biochem J.* 356, 461–471.
- (12) Zhang, W. et al. (2000) *J. Biol. Chem.* 275, 23355–23361.



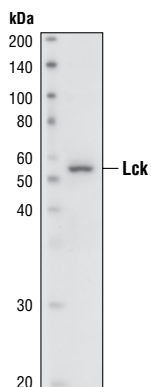
Western blot analysis of extracts from Jurkat cells treated with either H_2O_2 (2mM for 2 minutes) or with λ phosphatase; and extracts from Ramos cells treated with anti-human IgM (12 μ g/ml for 2 minutes) using **Phospho-Zap-70 (Tyr319)/Syk (Tyr352) (65E4) Rabbit mAb #2717**.



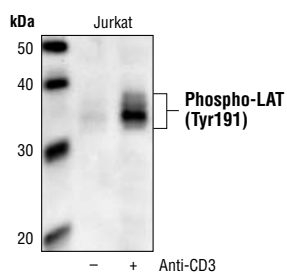
Western blot analysis of extracts from Jurkat and BW5147 cells, using **SLP-76 Antibody #4958**.



Western blot analysis of extracts from Jurkat cells, either untreated or treated with λ phosphatase, using **LAT Antibody #9166**.



Western blot analysis of Jurkat cell lysates, using **Lck (73A5) Rabbit mAb #2787**.



Western blot analysis of extracts from serum starved Jurkat cells, either untreated or anti-CD3 treated (10 μ g/ml for 2 minutes), using **Phospho-LAT (Tyr191) Antibody #3584**.

Western Immunoblotting Protocol (Primary Antibody Incubation in BSA)

For Western blots, incubate membrane with diluted antibody in 5% w/v BSA, 1X TBS, 0.1% Tween-20 at 4°C with gentle shaking, overnight.

A Solutions and Reagents

NOTE: Prepare solutions with Milli-Q or equivalently purified water.

- 1X Phosphate Buffered Saline (PBS)
- 1X SDS Sample Buffer:** 62.5 mM Tris-HCl (pH 6.8 at 25°C), 2% w/v SDS, 10% glycerol, 50 mM DTT, 0.01% w/v bromophenol blue or phenol red
- Transfer Buffer:** 25 mM Tris base, 0.2 M glycine, 20% methanol (pH 8.5)
- 10X Tris Buffered Saline (TBS):** To prepare 1 liter of 10X TBS: 24.2 g Tris base, 80 g NaCl; adjust pH to 7.6 with HCl (use at 1X).
- Nonfat Dry Milk (weight to volume [w/v])
- Blocking Buffer:** 1X TBS, 0.1% Tween-20 with 5% w/v nonfat dry milk; for 150 ml, add 15 ml 10X TBS to 135 ml water, mix. Add 7.5 g nonfat dry milk and mix well. While stirring, add 0.15 ml Tween-20 (100%).
- Wash Buffer:** 1X TBS, 0.1% Tween-20 (TBS/T)
- Bovine Serum Albumin (BSA)
- Primary Antibody Dilution Buffer:** 1X TBS, 0.1% Tween-20 with 5% BSA; for 20 ml, add 2 ml 10X TBS to 18 ml water, mix. Add 1.0 g BSA and mix well. While stirring, add 20 µl Tween-20 (100%).
- Phototope[®]-HRP Western Blot Detection System #7071:** Includes biotinylated protein ladder, secondary anti-rabbit (#7074) antibody conjugated to horseradish peroxidase (HRP), anti-biotin antibody conjugated to HRP, LumiGLO[®] chemiluminescent reagent and peroxide.
- Prestained Protein Marker, Broad Range (Premixed Format) #7720
- Biotinylated Protein Ladder Detection Pack #7727
- Blotting Membrane:** This protocol has been optimized for nitrocellulose membranes, which CST recommends. PVDF membranes may also be used.

B Protein Blotting

A general protocol for sample preparation is described below.

- Treat cells by adding fresh media containing regulator for desired time.
- Aspirate media from cultures; wash cells with 1X PBS; aspirate.
- Lyse cells by adding 1X SDS sample buffer (100 µl per well of 6-well plate or 500 µl per plate of 10 cm diameter plate). Immediately scrape the cells off the plate and transfer the extract to a microcentrifuge tube. Keep on ice.
- Sonicate for 10–15 seconds to shear DNA and reduce sample viscosity.
- Heat a 20 µl sample to 95–100°C for 5 minutes; cool on ice.
- Microcentrifuge for 5 minutes.
- Load 20 µl onto SDS-PAGE gel (10 cm x 10 cm).

NOTE: CST recommends loading prestained molecular weight markers (#7720, 10 µl/lane) to verify electrotransfer and biotinylated protein ladder (#7727, 10 µl/lane) to determine molecular weights.

- Electrotransfer to nitrocellulose or PVDF membrane.

C Membrane Blocking and Antibody Incubations

NOTE: Volumes are for 10 cm x 10 cm (100 cm²) of membrane; for different sized membranes, adjust volumes accordingly.

- (Optional) After transfer, wash nitrocellulose membrane with 25 ml TBS for 5 minutes at room temperature.
- Incubate membrane in 25 ml of blocking buffer for 1 hour at room temperature.
- Wash three times for 5 minutes each with 15 ml of TBS/T.
- Incubate membrane and primary antibody (at the appropriate dilution) in 10 ml primary antibody dilution buffer with gentle agitation overnight at 4°C.
- Wash three times for 5 minutes each with 15 ml of TBS/T.
- Incubate membrane with HRP-conjugated secondary antibody (1:2000) and HRP-conjugated anti-biotin antibody (1:1000) to detect biotinylated protein markers in 10 ml of blocking buffer with gentle agitation for 1 hour at room temperature.
- Wash three times for 5 minutes each with 15 ml of TBS/T.

D Detection of Proteins

- Incubate membrane with 10 ml LumiGLO[®] (0.5 ml 20X LumiGLO[®], 0.5 ml 20X Peroxide and 9.0 ml Milli-Q water) with gentle agitation for 1 minute at room temperature.

NOTE: LumiGLO[®] substrate can be further diluted if signal response is too fast.

- Drain membrane of excess developing solution (do not let dry), wrap in plastic wrap and expose to x-ray film. An initial 10-second exposure should indicate the proper exposure time.

NOTE: Due to the kinetics of the detection reaction, signal is most intense immediately following LumiGLO[®] incubation and declines over the following 2 hours.