Store at -20°C

T Cell Signaling Antibody Sampler Kit

#14541

1 Kit (8 x 20 µl)

Support: +1-978-867-2388 (U.S.)
www.cellsignal.com/support

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

Products Includedv	Product #	Quantity	Mol. Wt.	Isotype
CD3ε (CD3-12) Rat mAb	4443	20 µl	21 kDa	Rat IgG
Phospho-LAT (Tyr191) Antibody	3584	20 µl	36, 38 kDa	Rabbit
Phospho-Lck (Tyr505) Antibody	2751	20 µl	56 kDa	Rabbit
Phospho-PLCγ1 (Tyr783) (D6M9S) Rabbit mAb	14008	20 µl	155 kDa	Rabbit IgG
Phospho-SLP-76 (Ser376) (D9D6E) Rabbit mAb	14745	20 µl	76 kDa	Rabbit IgG
Phospho-Src Family (Tyr416) (D49G4) Rabbit mAb	6943	20 µl	60 kDa	Rabbit IgG
Phospho-Zap-70 (Tyr319)/Syk (Tyr352) (65E4) Rabbit mAb	2717	20 µl	70, 72 kDa	Rabbit IgG
Phospho-Zap-70 (Tyr493)/Syk (Tyr526) Antibody	2704	20 µl	70 kDa	Rabbit
Anti-rat IgG, HRP-linked Antibody	7077	100 µl		Goat
Anti-rabbit IgG, HRP-linked Antibody	7074	100 µl		Goat

For product specific protocols and a complete listing of recommended companion products please see the product web page at www.cellsignal.com

Storage: Supplied in 10 mM sodium HEPES (pH 7.5), 150 mM NaCl, 100 µg/ml BSA, 50% glycerol and less than 0.02% sodium azide. Store at –20°C. *Do not aliquot the antibody.*

Recommended Antibody Dilutions:

Western blotting	1:1000

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

Description: The T Cell Signaling Antibody Sampler Kit provides an economical means to investigate T cell receptor signaling. The kit contains enough antibody to perform two western blot experiments with each primary antibody.

Background: When T cells encounter antigens via the T cell receptor (TCR), information about the quantity and quality of antigens is relayed to the intracellular signal transduction machinery (1). This activation process depends mainly on CD3 (Cluster of Differentiation 3), a multiunit protein complex that directly associates with the TCR α and ß chains. CD3 is composed of four polypeptides: ζ, γ, ε and δ. Each of these polypeptides contains at least one immunoreceptor tyrosine-based activation motif (ITAM) (2). The Src family kinases Lck and Fyn are recruited to the TCR complex upon stimulation and activate the downstream tyrosine kinases to initiate signaling. Phosphorylation of Lck at Tyr394 leads to an increase in Lck activity while phosphorylation of Tyr505 in the Lck carboxy-terminal tail down-regulates Lck catalytic activity (3). Zap-70 and Syk are rapidly phosphorylated on several tyrosine residues through autophosphorylation and transphosphorylation by Src family tyrosine kinases. Activation loop phosphorylation of Zap-70 at Tyr493 and Syk at Tyr526 leads to complete activation of both kinases (4). Subsequent phosphorylation of other tyrosine residues within the kinase interdomain B region, including Zap-70 at Tyr315 and Zap-70 at Tyr 319, create docking sites for downstream signaling molecules. Zap-70 and Syk phosphorylate the transmembrane adaptor protein LAT at multiple, conserved tyrosine residues within SH2 binding motifs, exposing these motifs as docking sites for downstream signaling targets (5,6). The phosphorylation of LAT at Tyr171 and Tyr191 enables the binding of Grb2, Gads/SLP-76, PLCγ1, and PI3 kinase. The adapter protein SLP-76 is phosphorylated at Tyr113 and Tyr128, allowing for binding of the Grb2-like adapter Gads. Phosphorylation of SLP-76 at Ser376 by hematopoietic progenitor kinase 1 (HPK1) induces interaction with 14-3-3ε and down-regulates TCR signaling (7,8). Phosphoinositide-specific phospholipase PLCγ1 enzyme activity is also stimulated by Zap-70 and Syk phosphorylation on Tyr783, Tyr711, and Tyr1253, resulting in robust PI-4,5-P2 hydrolysis (9).

Specificity/Sensitivity: Unless otherwise indicated, each antibody will recognize endogenous total levels of target protein, and modification state antibodies will only recognize target proteins phosphorylated at the indicated residue. Phospho-Lck (Tyr505) Antibody may cross-react with certain phosphorylated Src family members due to high sequence homology. Phospho-Src Family (Tyr416) (D49G4) Rabbit mAb may cross-react with other Src family members (Lyn, Fyn, Lck, Yes and Hck) when phosphorylated at equivalent sites, and may cross react with overexpressed phosphorylated RTKs. Phospho-Zap-70 (Tyr319)/Syk (Tyr352) (65E4) Rabbit mAb cross-reacts with endogenous levels of Syk when phosphorylated at Tyr352. Phospho-Zap-70 (Tyr493)/Syk (Tyr526) Antibody cross-reacts with endogenous levels of Syk when phosphorylated at Tyr526.

Source/Purification: Monoclonal antibodies are produced by immunizing animals with a synthetic peptide corresponding to a region surrounding Pro184 of human CD3ε and with a synthetic phosphopeptide corresponding to residues surrounding Tyr783 of human PLCγ1 protein, Tyr416 of human Src protein, Ser376 of human SLP-76 protein, and Tyr319 of human Zap-70 protein. Polyclonal antibodies are produced by immunizing animals with a synthetic phosphopeptide corresponding to residues around Tyr191 of human LAT protein, Tyr505 of human Lck protein and, Tyr493 of human Zap-70 protein. Antibodies are purified by protein A and peptide affinity chromatography.

Background References:

(1) Kuhns, M.S. et al. (2006) *Immunity* 24, 133-9.
(2) Pitcher, L.A. and van Oers, N.S. (2003) *Trends Immunol* 24, 554-60.
(3) Chow, L.M. et al. (1993) *Nature* 365, 156-60.
(4) Wang, H. et al. (2010) *Cold Spring Harb Perspect Biol* 2, a002279.
(5) Zhang, W. et al. (1998) *Cell* 92, 83-92.
(6) Paz, P.E. et al. (2001) *Biochem J* 356, 461-71.
(7) Shui, J.W. et al. (2007) *Nat Immunol* 8, 84-91.
(8) Di Bartolo, V. et al. (2007) *J Exp Med* 204, 681-91.
(9) Beach, D. et al. (2007) *J Biol Chem* 282, 2937-46.

U. S. Patent No. 5,675,063

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Applications: **W**—Western **IP**—Immunoprecipitation **IHC**—Immunohistochemistry **ChIP**—Chromatin Immunoprecipitation **IF**—Immunofluorescence **F**—Flow cytometry **E-P**—ELISA-Peptide **Species Cross-Reactivity:** **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 **Ce**—C. elegans **Hr**—Horse **All**—all species expected Species enclosed in parentheses are predicted to react based on 100% homology.

Western Immunoblotting Protocol

For western blots, incubate membrane with diluted primary antibody in either 5% w/v BSA or nonfat dry milk, 1X TBS, 0.1% Tween® 20 at 4°C with gentle shaking, overnight.
NOTE: Please refer to primary antibody datasheet or product webpage for recommended primary antibody dilution buffer and recommended antibody dilution.

A. Solutions and Reagents

NOTE: Prepare solutions with reverse osmosis deionized (RODI) or equivalent grade water.

1. **20X Phosphate Buffered Saline (PBS):** (#9808) To prepare 1 L 1X PBS: add 50 ml 20X PBS to 950 ml dH_2O, mix.
2. **10X Tris Buffered Saline (TBS):** (#12498) To prepare 1 L 1X TBS: add 100 ml 10X to 900 ml dH_2O, mix.
3. **1X SDS Sample Buffer:** Blue Loading Pack (#7722) or Red Loading Pack (#7723) Prepare fresh 3X reducing loading buffer by adding 1/10 volume 30X DTT to 1 volume of 3X SDS loading buffer. Dilute to 1X with dH_2O.
4. **10X Tris-Glycine SDS Running Buffer:** (#4050) To prepare 1 L 1X running buffer: add 100 ml 10X running buffer to 900 ml dH_2O, mix.
5. **10X Tris-Glycine Transfer Buffer:** (#12539) To prepare 1 L 1X transfer buffer: add 100 ml 10X transfer buffer to 200 ml methanol + 700 ml dH_2O, mix.
6. **10X Tris Buffered Saline with Tween® 20 (TBST):** (#9997) To prepare 1 L 1X TBST: add 100 ml 10X TBST to 900 ml dH_2O, mix.
7. **Nonfat Dry Milk:** (#9999)
8. **Blocking Buffer:** 1X TBST with 5% w/v nonfat dry milk; for 150 ml, add 7.5 g nonfat dry milk to 150 ml 1X TBST and mix well.
9. **Wash Buffer:** (#9997) 1X TBST
10. **Bovine Serum Albumin (BSA):** (#9998)
11. **Primary Antibody Dilution Buffer:** 1X TBST with 5% BSA or 5% nonfat dry milk as indicated on primary antibody datasheet; for 20 ml, add 1.0 g BSA or nonfat dry milk to 20 ml 1X TBST and mix well.
12. **Biotinylated Protein Ladder Detection Pack:** (#7727)
13. **Prestained Protein Marker, Broad Range (Premixed Format):** (#7720)
14. **Blotting Membrane and Paper:** (#12369) This protocol has been optimized for nitrocellulose membranes. Pore size 0.2 µm is generally recommended.
15. **Secondary Antibody Conjugated to HRP:** anti-rabbit (#7074); anti-mouse (#7076)
16. **Detection Reagent:** LumiGLO® chemiluminescent reagent and peroxide (#7003) or SignalFire™ ECL Reagent (#6883)

B. Protein Blotting

A general protocol for sample preparation.

1. Treat cells by adding fresh media containing regulator for desired time.
2. Aspirate media from cultures; wash cells with 1X PBS; aspirate.
3. Lyse cells by adding 1X SDS sample buffer (100 µl per well of 6-well plate or 500 µl for a 10 cm diameter plate). Immediately scrape the cells off the plate and transfer the extract to a microcentrifuge tube. Keep on ice.
4. Sonicate for 10–15 sec to complete cell lysis and shear DNA (to reduce sample viscosity).
5. Heat a 20 µl sample to 95–100°C for 5 min; cool on ice.
6. Microcentrifuge for 5 min.
7. Load 20 µl onto SDS-PAGE gel (10 cm x 10 cm). **NOTE:** Loading of prestained molecular weight markers (#7720, 10 µl/lane) to verify electrotransfer and biotinylated protein ladder (#7727, 10 µl/lane) to determine molecular weights are recommended.
8. Electrotransfer to nitrocellulose membrane (#12369).

C. Membrane Blocking and Antibody Incubations

NOTE: Volumes are for 10 cm x 10 cm (100 cm^2) of membrane; for different sized membranes, adjust volumes accordingly.

I. Membrane Blocking

1. (Optional) After transfer, wash nitrocellulose membrane with 25 ml TBS for 5 min at room temperature.
2. Incubate membrane in 25 ml of blocking buffer for 1 hr at room temperature.
3. Wash three times for 5 min each with 15 ml of TBST.

II. Primary Antibody Incubation

1. Incubate membrane and primary antibody (at the appropriate dilution and diluent as recommended in the product datasheet) in 10 ml primary antibody dilution buffer with gentle agitation overnight at 4°C.
2. Wash three times for 5 min each with 15 ml of TBST.
3. Incubate membrane with the species appropriate HRP-conjugated secondary antibody (#7074 or #7076 at 1:2000) and anti-biotin, HRP-linked Antibody (#7075 at 1:1000–1:3000) to detect biotinylated protein markers in 10 ml of blocking buffer with gentle agitation for 1 hr at room temperature.
4. Wash three times for 5 min each with 15 ml of TBST.
5. Proceed with detection (Section D).

D. Detection of Proteins

1. Incubate membrane with 10 ml LumiGLO® (0.5 ml 20X LumiGLO® #7003, 0.5 ml 20X peroxide, and 9.0 ml purified water) or 10 ml SignalFire™ #6883 (5 ml Reagent A, 5 ml Reagent B) with gentle agitation for 1 min at room temperature.
2. Drain membrane of excess developing solution (do not let dry), wrap in plastic wrap and expose to x-ray film. An initial 10 sec exposure should indicate the proper exposure time. **NOTE:** Due to the kinetics of the detection reaction, signal is most intense immediately following incubation and declines over the following 2 hr.