Revision 1

#14541 Store at -20°C

# T Cell Signaling Antibody Sampler Kit

1 Kit (8 x 20 microliters)

Cell Signaling TECHNOLOGY®

**Orders:** 877-616-CELL (2355)
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**Support:** 877-678-TECH (8324)

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**For Research Use Only. Not for Use in Diagnostic Procedures.**

| Product Includes | Product # | Quantity | Mol. Wt | Isotype/Source |
|---|---|---|---|---|
| CD3ε (CD3-12) Rat mAb | 4443 | 20 µl | 21 kDa | Rat IgG1 |
| Phospho-LAT (Tyr220) 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-Src Family (Tyr416) (D49G4) Rabbit mAb | 6943 | 20 µl | 60 kDa | Rabbit IgG |
| Phospho-SLP-76 (Ser376) (D9D6E) Rabbit mAb | 14745 | 20 µl | 76 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 |

Please visit cellsignal.com for individual component applications, species cross-reactivity, dilutions, protocols, and additional product information.

## Description

The T Cell Signaling Antibody Sampler Kit provides an economical means to investigate T cell receptor signaling. The kit contains primary and secondary antibodies to perform two western blot experiments per primary antibody.

## 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.

## 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 Tyr220 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).

## 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.

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## Limited Uses