# #9768 Store at $-20^{\circ}$ C

# **B Cell Signaling Antibody Sampler Kit**



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1 Kit (8 x 20 microliters)

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

Product Includes	Product #	Quantity	Mol. Wt	Isotype/Source
Phospho-Src Family (Tyr416) (D49G4) Rabbit mAb	6943	20 μΙ	60 kDa	Rabbit IgG
Phospho-Syk (Tyr525/526) (C87C1) Rabbit mAb	2710	20 μΙ	72 kDa	Rabbit IgG
Phospho-Btk (Tyr223) (D1D2Z) Rabbit mAb	87457	20 μΙ	78 kDa	Rabbit IgG
Phospho-PLCy2 (Tyr759) (E9E9Y) Rabbit mAb	50535	20 μΙ	150 kDa	Rabbit IgG
Phospho-BLNK (Tyr96) Antibody	3601	20 μΙ	68, 70 kDa	Rabbit
Phospho-CD19 (Tyr531) Antibody	3571	20 μΙ	95 kDa	Rabbit
Phospho-CD79A (Tyr182) Antibody	5173	20 μΙ	45-55 kDa	Rabbit
CD79A (D1X5C) XP <sup>®</sup> Rabbit mAb	13333	20 μΙ	45-55 kDa	Rabbit IgG
Anti-rabbit IgG, HRP-linked Antibody	7074	100 μΙ		Goat

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

### **Description**

The B Cell Signaling Antibody Sampler Kit provides an economical means to examine key signaling proteins commonly associated with B cell activation. The provided antibodies allow monitoring of both total protein levels and the phosphorylation state. The kit includes enough primary and secondary antibody to perform two western mini-blot experiments.

### Storage

### **Background**

Supplied in 10 mM sodium HEPES (pH 7.5), 150 mM NaCl, 100  $\mu$ g/ml BSA, 50% glycerol and less than 0.02% sodium azide. Store at  $-20^{\circ}$ C. Do not aliquot the antibody.

Antigen receptors found on the surface of B cells contain a heterodimeric signaling component composed of CD79A and CD79B, also known as Ig  $\alpha$  and Ig  $\beta$ , respectively. Presence of this receptor complex is essential for B-cell development and function. Antigen binding precedes formation of the CD79A and CD79B heterodimer and subsequent activation of receptor associated kinases. Tyr182 of mouse CD79A (corresponding to Tyr188 of human CD79A) is one of two key tyrosine residues in the immunoreceptor tyrosine-based activation motif (ITAM) of CD79A that are phosphorylated by Src family kinases (e.g., Lyn, Blk), and play a critical role in modulating signal transduction following immune receptor activation.

Syk is a protein tyrosine kinase that plays an important role in intracellular signal transduction in hematopoietic cells (1-3). Syk interacts with immunoreceptor tyrosine-based activation motifs (ITAMs) located in the cytoplasmic domains of immune receptors (4). It couples the activated immunoreceptors to downstream signaling events that mediate diverse cellular responses, including proliferation, differentiation, and phagocytosis (4). There is also evidence that Syk plays a role in nonimmune cells; Syk is a potential tumor suppressor in human breast carcinomas (5). Tyrosine 525 and 526 are located in the activation loop of the Syk kinase domain, and phosphorylation of Tyr525/526 of human Syk (equivalent to the Tyr519/520 of mouse Syk) is essential for Syk function (6).

Lyn, one of the Src family members, is predominantly expressed in hematopoietic cells (7). Two tyrosine residues have been reported to play a crucial role in the regulation of protein tyrosine kinases of the Src family. Autophosphorylation of Tyr396 (equivalent to Tyr416 of Src), located in the catalytic domain, correlates with enzyme activation. Csk-mediated phosphorylation of the carboxy-terminal Tyr507 (equivalent to Tyr527 of Src) inactivates the kinase. Tyrosine phosphorylation and activation of Lyn occurs upon association with cell surface receptors such as the B cell Ag receptor (BCR) and CD40 (8-10).

Bruton's tyrosine kinase (Btk) is a member of the Btk/Tec family of cytoplasmic tyrosine kinases. Btk plays an important role in B cell development (11,12). Activation of B cells by various ligands is accompanied by Btk membrane translocation mediated by its PH domain binding to phosphatidylinositol-3,4,5-trisphosphate (13-15). The membrane-located Btk is active and associated with transient phosphorylation of two tyrosine residues, Tyr551 and Tyr223. Tyr551 in the activation loop is transphosphorylated by the Src family tyrosine kinase, leading to autophosphorylation at Tyr223 within the SH3 domain, which is necessary for full activation (16,17).

CD19 is a 95 kDa coreceptor that amplifies the signaling cascade in B cells (18). On the B cell surface, CD19 associates with CD21, CD81, and Leu-13 to exert its function. The cytoplasmic tail of CD19 has nine conserved tyrosine residues playing critical roles in CD19-mediated function by coupling signaling molecules to the receptor (18). After BCR or CD19 ligation, Tyr531 and Tyr500 of CD19 are progressively phosphorylated. This phosphorylation enables the coupling of PI3 kinase and Src family tyrosine kinase to CD19 and activates the PI3K and Src signaling pathways (19,20).

B cell linker protein (BLNK), also known as SLP-65 or BASH, is an adaptor molecule that plays key roles in B cell activation and B cell antigen receptor (BCR) engagement. BLNK acts at the interface between BCR-associated Syk and downstream signaling cascades

Phosphoinositide-specific phospholipase C (PLC) plays a significant role in transmembrane signaling. PLCgamma2 is engaged in antigen-dependent signaling in B cells. Phosphorylation by Btk or Lck at tyrosines 753, 759, 1197 and 1217 is correlated with PLCgamma2 activity.

### Background References

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