

B Cell Signaling Antibody Sampler Kit



✓ 1 Kit
(8 x 20 µl)

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

Products Included	Product #	Quantity	Mol. Wt.	Isotype
Phospho-BLNK (Tyr96) Antibody	3601	20 µl	68, 70 kDa	Rabbit IgG
Phospho-Syk (Tyr525/526) (C87C1) Rabbit mAb	2710	20 µl	72 kDa	Rabbit IgG
Phospho-PLCγ2 (Tyr759) Antibody	3874	20 µl	150 kDa	Rabbit IgG
Phospho-CD79A (Tyr182) Antibody	5173	20 µl	45-55 kDa	Rabbit IgG
Phospho-Src Family (Tyr416) (D49G4) Rabbit mAb	6943	20 µl	60 kDa	Rabbit IgG
Phospho-Btk (Tyr223) Antibody	5082	20 µl	77 kDa	Rabbit IgG
CD79A Antibody	3351	20 µl	45-55 kDa	Rabbit IgG
Phospho-CD19 (Tyr531) Antibody	3571	20 µl	95 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 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 antibody to perform two western blot experiments with each primary antibody.

Background: Antigen receptors found on the surface of B cells contain a heterodimeric signaling component composed of CD79A and CD79B, also known as Ig α and Ig β, 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, Btk), 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).

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 validation data and a complete listing of recommended companion products.

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. PLCγ2 is engaged in antigen-dependent signaling in B cells. Phosphorylation by Btk or Lck at tyrosines 753, 759, 1197 and 1217 is correlated with PLCγ2 activity.

Specificity/Sensitivity: All antibodies contained in this kit detect endogenous levels of their respective target protein.

Source/Purification: Polyclonal antibodies are produced by immunizing animals with a synthetic peptide and are purified by protein A and peptide affinity chromatography. Monoclonal antibodies are produced by immunizing animals with recombinant human proteins or synthetic peptides.

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

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—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.

Background References:

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- (4) Turner, M. et al. (2000) *Immunol Today* 21, 148-54.
- (5) Coopman, P.J. et al. (2000) *Nature* 406, 742-7.
- (6) Zhang, J. et al. (2000) *J Biol Chem* 275, 35442-7.
- (7) Yamanashi, Y. et al. (1989) *Proc Natl Acad Sci USA* 86, 6538-42.
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- (11) Khan, W.N. (2001) *Immunol Res* 23, 147-56.
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- (13) Salim, K. et al. (1996) *EMBO J* 15, 6241-50.
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- (16) Rawlings, D.J. et al. (1996) *Science* 271, 822-5.
- (17) Park, H. et al. (1996) *Immunity* 4, 515-25.
- (18) Tedder, T.F. et al. (1997) *Immunity* 6, 107-18.
- (19) Buhl, A.M. and Cambier, J.C. (1999) *J Immunol* 162, 4438-46.
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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.

- 20X Phosphate Buffered Saline (PBS):** (#9808) To prepare 1 L 1X PBS: add 50 ml 20X PBS to 950 ml dH₂O, mix.
- 10X Tris Buffered Saline (TBS):** (#12498) To prepare 1 L 1X TBS: add 100 ml 10X to 900 ml dH₂O, mix.
- 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 dH₂O.
- 10X Tris-Glycine SDS Running Buffer:** (#4050) To prepare 1 L 1X running buffer: add 100 ml 10X running buffer to 900 ml dH₂O, mix.
- 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 dH₂O, mix.
- 10X Tris Buffered Saline with Tween® 20 (TBST):** (#9997) To prepare 1 L 1X TBST: add 100 ml 10X TBST to 900 ml dH₂O, mix.
- Nonfat Dry Milk:** (#9999)
- 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.
- Wash Buffer:** (#9997) 1X TBST
- Bovine Serum Albumin (BSA):** (#9998)
- 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.
- Biotinylated Protein Ladder Detection Pack:** (#7727)
- Prestained Protein Marker, Broad Range (Premixed Format):** (#7720)
- Blotting Membrane and Paper:** (#12369) This protocol has been optimized for nitrocellulose membranes. Pore size 0.2 µm is generally recommended.
- Secondary Antibody Conjugated to HRP:** anti-rabbit (#7074); anti-mouse (#7076)
- Detection Reagent:** LumiGLO® chemiluminescent reagent and peroxide (#7003) or SignalFire™ ECL Reagent (#6883)

B. Protein Blotting

A general protocol for sample preparation.

- 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 for a 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 sec to complete cell lysis and shear DNA (to reduce sample viscosity).
- Heat a 20 µl sample to 95–100°C for 5 min; cool on ice.
- Microcentrifuge for 5 min.
- 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.
- Electrotransfer to nitrocellulose membrane (#12369).

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.

I. Membrane Blocking

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

II. Primary Antibody Incubation

- 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.
- Wash three times for 5 min each with 15 ml of TBST.
- 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.
- Wash three times for 5 min each with 15 ml of TBST.
- Proceed with detection (Section D).

D. Detection of Proteins

- 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.
- 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.