

Phospho-Syk Antibody Sampler Kit

1 Kit
 (4 x 20 µl)



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

Products Included	Product #	Quantity	Mol. Wt.	Source
Phospho-Syk (Tyr323) Antibody	2715	20 µl	72 kDa	Rabbit IgG
Syk (D3Z1E) XP® Rabbit mAb	13198	20 µl	72 kDa	Rabbit IgG
Phospho-Syk (Tyr525/526) (C87C1) Rabbit mAb	2710	20 µl	72 kDa	Rabbit IgG
Phospho-Zap-70 (Tyr319)/Syk (Tyr352) Antibody	2701	20 µl	70 kDa (Zap-70), 72 kDa (Syk)	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.

Background: Syk, a protein tyrosine kinase, is widely expressed and 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 of a role for Syk in nonimmune cells, and Syk is a potential tumor suppressor in human breast carcinomas (5). Tyr323 is a negative regulatory phosphorylation site within the SH2-kinase linker region in Syk. Phosphorylation of Tyr323 provides a direct binding site to the TKB domain of Cbl (6,7). Tyrosine 352 of Syk is involved in the association of PLC-γ1 (8). Tyrosines 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 (9).

Description: Phospho-Syk Sampler Kit provides an economical means to evaluate the activation status of

Syk, including the phosphorylation of Tyr323, Tyr352 and Tyr525/526. The control Syk Antibody is also included. The kit contains enough primary and secondary antibodies for two mini-blot experiments.

Specificity/Sensitivity: Phospho-Syk (Tyr525/526) (C87C1) Rabbit mAb detects endogenous levels of Syk protein only when phosphorylated at Tyr525/526 of human Syk or Tyr519/520 of mouse Syk. It also detects Syk protein when singly phosphorylated at Tyr526 of human Syk or Tyr520 of mouse Syk. Phospho-Syk (Tyr323) Antibody detects endogenous levels of Syk only when phosphorylated at Tyr323. Syk (D3Z1E) XP® Rabbit mAb recognizes endogenous levels of total Syk protein. All other antibodies in the kit do not cross-react with phosphorylated or nonphosphorylated forms of other related family members.

Source/Purification: Polyclonal antibodies are produced by immunizing animals with synthetic phosphopeptides corresponding to residues surrounding Tyr323 of human Syk or Tyr319 of human Zap-70. Polyclonal 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 antibody.

Recommended Antibody Dilutions:
Western blotting 1:1000

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

Monoclonal antibody is produced by immunizing animals with a synthetic peptide corresponding to residues around Asn463 of human Syk protein or residues surrounding Tyr525/526 of human Syk.

Background References:

- (1) Cheng, A.M. and Chan, A.C. (1997) *Curr. Opin. Immunol.* 9, 528-533.
- (2) Kurosaki, T. et al. (1997) *Curr. Opin. Immunol.* 9, 309-318.
- (3) Chu, D.H. et al. (1998) *Immunol. Rev.* 165, 167-180.
- (4) Turner, M. et al. (2000) *Immunol. Today* 21, 148-154.
- (5) Coopman, P.J. et al. (2000) *Nature* 406, 742-747.
- (6) Decker, M. et al. (1998) *J. Biol. Chem.* 273, 8867-8874.
- (7) Rao, N. et al. (2001) *EMBO J.* 20, 7085-7095.
- (8) Law, C.L. et al. (1996) *Mol. Cell. Biol.* 16, 1305-1315.

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

Applications Key: W—Western IP—Immunoprecipitation IHC—Immunohistochemistry IC—Immunocytochemistry IF—Immunofluorescence F—Flow cytometry E—ELISA D—DELFIA®
Species Cross-Reactivity Key: H—human M—mouse R—rat Hm—hamster Mk—monkey Mi—mink C—chicken X—Xenopus Z—zebra fish B—bovine All—all species expected
 Species enclosed in parentheses are predicted to react based on 100% sequence 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.

- 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.