

**#2712** Store at -20°C

# Syk Antibody



100 µl  
 (10 Western mini-blot)

**Orders** ■ 877-616-CELL (2355)  
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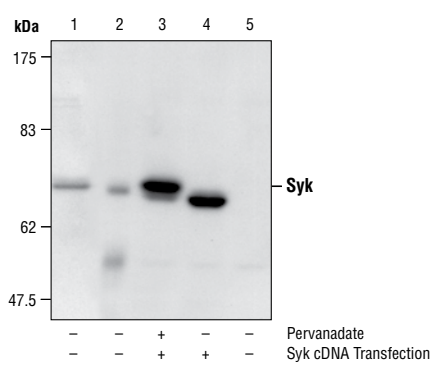
This product is for *in vitro* research use only and is not intended for use in humans or animals.

Applications	Species Cross-Reactivity*		Molecular Wt. 72 kDa	Source Rabbit
	W	H, M		

**Background:** 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 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).

**Specificity/Sensitivity:** Syk Antibody detects endogenous levels of total Syk protein. It does not cross-react with other members of the Syk/Zap-70 tyrosine kinase family.

**Source/Purification:** Polyclonal antibodies are produced by immunizing rabbits with a synthetic peptide (KLH-coupled) corresponding to the carboxy-terminal sequence of human Syk. Antibodies are purified by protein A and peptide affinity chromatography.



Western blot analysis of extracts from Jurkat (lane 1) and THP1 (lane 2) cells, and untransfected or Syk cDNA-transfected DT40 cells, treated as indicated (lanes 3-5), using Syk Antibody.

**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.
- (9) Zhang, J. et al. (2000) *J. Biol. Chem.* 275, 35442–35447.

**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.

**\*Species cross-reactivity is determined by Western blot.**

**Recommended Antibody Dilutions:**  
Western blotting 1:1000

**Companion Products:**  
 Phospho-Syk (Tyr525/526) Antibody #2711  
 Phototope®-HRP Western Blot Detection System, Anti-rabbit IgG, HRP-linked Antibody #7071  
 Anti-rabbit IgG, HRP-linked Antibody #7074  
 Prestained Protein Marker, Broad Range (Premixed Format) #7720  
 Biotinylated Protein Ladder Detection Pack #7727  
 20X LumiGLO® Reagent and 20X Peroxide #7003

**IMPORTANT: For Western blots, incubate membrane with diluted antibody in 5% w/v BSA, 1X TBS, 0.1% Tween-20 at 4°C with gentle shaking, overnight.**

**Applications Key:** W—Western IP—Immunoprecipitation IHC—Immunohistochemistry IC—Immunocytochemistry IF—Immunofluorescence  
**Species Cross-Reactivity Key:** H—human M—mouse R—rat Hm—hamster Mk—monkey Mi—mink C—chicken X—Xenopus  
 F—Flow cytometry E—ELISA D—DELFIATM  
 Z—zebra fish B—bovine All—all species expected

## Western Immunoblotting Protocol (Primary Antibody Incubation in BSA)

For Western blots, incubate membrane with diluted antibody in 5% w/v BSA, 1X TBS, 0.1% Tween-20 at 4°C with gentle shaking, overnight.

### A Solutions and Reagents

**NOTE:** Prepare solutions with Milli-Q or equivalently purified water.

- 1X Phosphate Buffered Saline (PBS)
- 1X SDS Sample Buffer:** 62.5 mM Tris-HCl (pH 6.8 at 25°C), 2% w/v SDS, 10% glycerol, 50 mM DTT, 0.01% w/v bromophenol blue or phenol red
- Transfer Buffer:** 25 mM Tris base, 0.2 M glycine, 20% methanol (pH 8.5)
- 10X Tris Buffered Saline (TBS):** To prepare 1 liter of 10X TBS: 24.2 g Tris base, 80 g NaCl; adjust pH to 7.6 with HCl (use at 1X).
- Nonfat Dry Milk (weight to volume [w/v])
- Blocking Buffer:** 1X TBS, 0.1% Tween-20 with 5% w/v nonfat dry milk; for 150 ml, add 15 ml 10X TBS to 135 ml water, mix. Add 7.5 g nonfat dry milk and mix well. While stirring, add 0.15 ml Tween-20 (100%).
- Wash Buffer:** 1X TBS, 0.1% Tween-20 (TBS/T)
- Bovine Serum Albumin (BSA)
- Primary Antibody Dilution Buffer:** 1X TBS, 0.1% Tween-20 with 5% BSA; for 20 ml, add 2 ml 10X TBS to 18 ml water, mix. Add 1.0 g BSA and mix well. While stirring, add 20 µl Tween-20 (100%).
- Phototope<sup>®</sup>-HRP Western Blot Detection System #7071:** Includes biotinylated protein ladder, secondary anti-rabbit (#7074) antibody conjugated to horseradish peroxidase (HRP), anti-biotin antibody conjugated to HRP, LumiGLO<sup>®</sup> chemiluminescent reagent and peroxide.
- Prestained Protein Marker, Broad Range (Premixed Format) #7720
- Biotinylated Protein Ladder Detection Pack #7727
- Blotting Membrane:** This protocol has been optimized for nitrocellulose membranes, which CST recommends. PVDF membranes may also be used.

### B Protein Blotting

A general protocol for sample preparation is described below.

- 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 per plate of 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 seconds to shear DNA and reduce sample viscosity.
- Heat a 20 µl sample to 95–100°C for 5 minutes; cool on ice.
- Microcentrifuge for 5 minutes.
- Load 20 µl onto SDS-PAGE gel (10 cm x 10 cm).

**NOTE:** CST recommends loading prestained molecular weight markers (#7720, 10 µl/lane) to verify electrotransfer and biotinylated protein ladder (#7727, 10 µl/lane) to determine molecular weights.

- Electrotransfer to nitrocellulose or PVDF membrane.

### C Membrane Blocking and Antibody Incubations

**NOTE:** Volumes are for 10 cm x 10 cm (100 cm<sup>2</sup>) of membrane; for different sized membranes, adjust volumes accordingly.

- (Optional) After transfer, wash nitrocellulose membrane with 25 ml TBS for 5 minutes at room temperature.
- Incubate membrane in 25 ml of blocking buffer for 1 hour at room temperature.
- Wash three times for 5 minutes each with 15 ml of TBS/T.
- Incubate membrane and primary antibody (at the appropriate dilution) in 10 ml primary antibody dilution buffer with gentle agitation overnight at 4°C.
- Wash three times for 5 minutes each with 15 ml of TBS/T.
- Incubate membrane with HRP-conjugated secondary antibody (1:2000) and HRP-conjugated anti-biotin antibody (1:1000) to detect biotinylated protein markers in 10 ml of blocking buffer with gentle agitation for 1 hour at room temperature.
- Wash three times for 5 minutes each with 15 ml of TBS/T.

### D Detection of Proteins

- Incubate membrane with 10 ml LumiGLO<sup>®</sup> (0.5 ml 20X LumiGLO<sup>®</sup>, 0.5 ml 20X Peroxide and 9.0 ml Milli-Q water) with gentle agitation for 1 minute at room temperature.

**NOTE:** LumiGLO<sup>®</sup> substrate can be further diluted if signal response is too fast.

- Drain membrane of excess developing solution (do not let dry), wrap in plastic wrap and expose to x-ray film. An initial 10-second exposure should indicate the proper exposure time.

**NOTE:** Due to the kinetics of the detection reaction, signal is most intense immediately following LumiGLO<sup>®</sup> incubation and declines over the following 2 hours.