

Src Antibody Sampler Kit



✓ 1 Kit
(5 x 20 µl)

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

Products Included	Product #	Quantity	Mol. Wt.	Isotype
Non-phospho-Src (Tyr527) Antibody	2107	20 µl	60 kDa	Rabbit IgG
Non-phospho-Src (Tyr416) (7G9) Mouse mAb	2102	20 µl	60 kDa	Mouse IgG2b
Phospho-Src (Tyr527) Antibody	2105	20 µl	60 kDa	Rabbit IgG
Phospho-Src Family (Tyr416) (D49G4) Rabbit mAb	6943	20 µl	60 kDa	Rabbit IgG
Src (32G6) Rabbit mAb	2123	20 µl	60 kDa	Rabbit IgG
Anti-rabbit IgG, HRP-linked Antibody	7074	100 µl		Goat
Anti-mouse IgG, HRP-linked Antibody	7076	100 µl		Goat

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

See www.cellsignal.com for individual component applications, species cross-reactivity, dilutions, and additional application protocols.

Description: The Src Antibody Sampler kit provides an economical means of evaluating total Src protein levels and its phosphorylation status. The kit includes enough antibody to perform two western blot experiments with each primary antibody.

Background: The Src family of protein tyrosine kinases, which includes Src, Lyn, Fyn, Yes, Lck, Blk, and Hck, are important in the regulation of growth and differentiation of eukaryotic cells (1). Src activity is regulated by tyrosine phosphorylation at two sites, but with opposing effects. While phosphorylation at Tyr416 in the activation loop of the kinase domain upregulates enzyme activity, phosphorylation at Tyr527 in the carboxy-terminal tail by Csk renders the enzyme less active (2).

Lyn is a member of the Src family that is predominantly expressed in hematopoietic cells (3). Lyn participates in signaling from multiple cell surface receptors such as the B cell Ag receptor (BCR) and CD40 (4).

Lck is essential for T-lymphocyte activation and differentiation (5,6). The activity of Lck is regulated by protein kinases and phosphatases. Phosphorylation of the C-terminal tyrosine 505 serves to downregulate Lck catalytic activity, while phosphorylation at tyrosine 394 leads to an increase in Lck activity (7).

Specificity/Sensitivity: Each antibody in the kit detects endogenous levels of Src only when in the indicated phosphorylation state at the indicated residue. The antibodies may cross-react with other Src family members when in equivalent phosphorylation states.

Source/Purification: Polyclonal antibodies are produced by immunizing animals with synthetic phospho- or non-phosphopeptides corresponding to residues surrounding Tyr527 of human Src protein. The mouse monoclonal antibody is produced by immunizing animals with a synthetic non-phosphopeptide corresponding to residues surrounding Tyr416 of human Src protein. The rabbit monoclonal antibody for P-Src Y416 is produced

by immunizing animals with a synthetic phosphopeptide corresponding to residues surrounding Tyr416 of human Src protein. The Src (32G6) rabbit monoclonal antibody is produced by immunizing animals with a synthetic peptide corresponding to residues near the amino-terminus of human Src. Polyclonal antibodies are purified by protein A and peptide affinity chromatography.

Background References:

- (1) Thomas, S.M. and Brugge, J.S. (1997) *Annu. Rev. Cell Dev. Biol.* 13, 513–609.
- (2) Hunter, T. (1987) *Cell* 49, 1–4.
- (3) Yamanashi, Y. et al. (1989) *Proc. Natl. Acad. Sci. USA* 86, 6538–6542.
- (4) Yamanashi, Y. et al. (1991) *Science* 251, 192–194.
- (5) Molina, T. J. et al. (1992) *Nature* 357, 161–164.
- (6) Straus, D. B. et al. (1992) *Cell* 70, 585–593.
- (7) Chow, L. M. et al. (1993) *Nature* 365, 156–160.

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

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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.

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.