

Anti-phospho-Src (Tyr416), clone 9A6

Monoclonal Antibody

Cat. # 05-677

Lot # DAM1554320

pack size: 100 µg

Store at -20°C

FOR RESEARCH USE ONLY



Certificate of Analysis

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Applications	Species Cross-Reactivity	Antibody Isotype	Epitope/Region	Host Species	Molecular Weight	Accession #
WB, IP	H, M, R	IgG1k	Tyr416	M	60 kDa	NM_005417

Background

Src (also known as pp60src) is a non receptor Tyrosine Kinase involved in signal transduction in many biological systems and implicated in the development of human tumors. There are two critical phosphorylation sites of tyrosine on Src, tyrosine 418 and tyrosine 529 (referring to human Src sequence). The tyrosine 418 is located in the catalytic domain and is one of the autophosphorylation sites. Full catalytic activity of Src requires phosphorylation of tyrosine 418. This region of Src is also highly conserved in all of the related Src-family kinases, and thus prior immunoprecipitation may be required to identify which Src family member is being activated.

Presentation

Purified mouse monoclonal IgG1k lyophilized from 1 mL 2x PBS / 0.09 % Na-azide / PEG and Sucrose. Reconstitute with 100 µL H₂O (15 min, RT) for a final concentration of 1 mg/mL. Lyophilized. Store at -20°C.

Rehydration: Rehydrate with 100 µL of sterile, distilled water to make a 1 mg/mL solution.

Specificity

Recognizes phospho-Src, Mr 60kDa. The antibody does not crossreact with the nonphosphorylated form of Src or with unrelated phosphorylation sites. Predicted to recognize all Src family members phosphorylated at the tyrosine corresponding to Tyr416 of avian Src.

Immunogen

KLH-conjugated, synthetic peptide containing phosphotyrosine in the sequence context corresponding to tyrosine 416 of avian Src. Clone 9A6.

Molecular Weight

60 kDa

Method of Purification

Thiophilic adsorption and size exclusion chromatography

Storage and Handling

Lyophilized: stable for 1 year at -20°C from date of receipt. Rehydrated: Stable for 3 months at 2-8°C. Aliquot solution to store frozen and avoid repeated freeze thaw cycles.

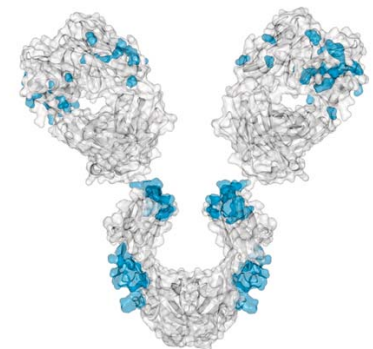
Control

Chick embryo fibroblast (CEF) cells expressing human wild-type Src protein

Quality Control Testing

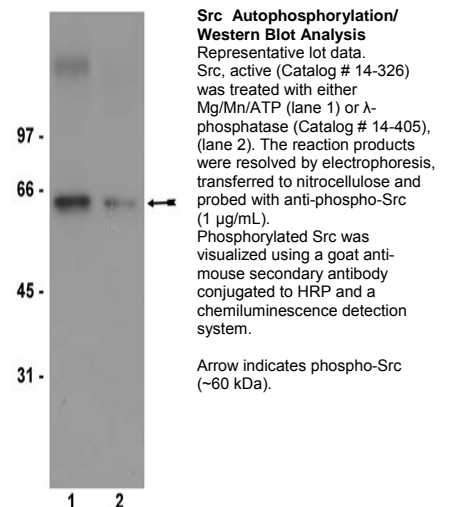
Evaluated by immunoprecipitating Src from 3T3 cell lysates with anti-Src (Catalog #05-184), but not after phosphatase treatment with YOP.

Src Autophosphorylation/Western Blotting Analysis: 0.5-2 µg/mL of this antibody strongly detected recombinant Src (Catalog # 14-326) after incubation with Mg/Mn/ATP. Phospho-specificity was confirmed by dephosphorylation of the Src protein with λ-phosphatase.



References

1. Katyal, S. and Godbout, R. (2004). *Embo J.* 23: 1878-88.



APPLICATION LEGEND: WB Western Blotting IP Immunoprecipitation IC Immunocytochemistry IF Immunofluorescence
IH Immunohistochemistry (Tissue)

SPECIES LEGEND: H Human M Mouse R Rat Rb Rabbit WR Most Common Vertebrates

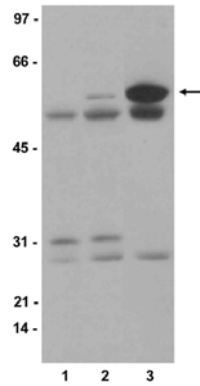
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Additional Research Applications

Immunoprecipitation/Immunoblot Analysis: 4 µg/mL of a previous lot detected phospho-Src immunoprecipitated from 3T3 cell lysates with 10 µg of anti-Src (Catalog # 05-184), but not after phosphatase treatment with YOP.



Western Blot Analysis

Representative lot data. Src Immunoprecipitated with anti-Src (05-184) was either treated (lane 1) or untreated (lanes 2 and 3) with the phosphotyrosine phosphatase YOP (14-229), then probed with anti-phospho-Src (lanes 1 & 2), or anti-Src (05-184) (lane 3).

Arrow indicates phospho-Src (~60 kDa).

PROTOCOL

Western Blot

1. Label two microcentrifuge tubes, one plus (+) for λ-phosphatase treatment and one minus (-) for no λ-phosphatase treatment.
2. Prepare each tube as described in the following table:

	λ-phosphatase (-)	λ-phosphatase (+)
Src Manganese/ATP Cocktail (Catalog # 20-110)	12.5µl	-
Src Kinase Reaction Buffer (Catalog # 20-121)	12.5µl	12.5µl
Src, active (Catalog # 14-326)	5µl (500ng)	5µl
sterile, distilled water	20µl	21.5µl
DTT (Catalog # 20-265)	-	1µl
Lambda Phosphatase (Catalog # 14-405)	-	10µl (750U)

3. Incubate the tubes for 15 minutes at 30°C with constant agitation.
4. Stop the reaction by adding 3 volumes of 2X Laemmli sample buffer.
5. Heat for 10 minutes at 95°C.
6. Perform SDS-PAGE on 20 µL (50 ng of Src) samples from each tube.
7. Transfer the gel to nitrocellulose and wash twice with water.
8. Block the blotted nitrocellulose in freshly prepared TBS containing 3% nonfat dry milk (Catalog # 20-200) and 0.05% Tween 20 (TBST-MLK) for 20 minutes at room temperature with constant agitation.
9. Incubate the nitrocellulose with 0.5-2 µg/mL of anti-phospho-Src (Tyr416), clone 9A6, diluted in freshly prepared TBST-MLK overnight with agitation at 4°C or for 2 hours at room temperature.
10. Wash the nitrocellulose twice with water.
11. Incubate the nitrocellulose in the secondary reagent of choice (a goat anti-mouse HRP conjugated IgG, Catalog # 12-349, 1:3000 dilution was used) in TBST-MLK for 1.5 hours with agitation at room temperature.
12. Wash the nitrocellulose twice with water.
13. Wash the nitrocellulose in TBS-0.05% Tween 20 for 3-5 minutes.
14. Rinse the nitrocellulose in 4-5 changes of water.
15. Use detection method of choice (enhanced chemiluminescence was used).

Immunoprecipitation

1. Add 10 µg of anti-phospho-Src (Tyr416), clone 9A6 and 60 µL (30 µL packed beads) of washed Protein G agarose bead slurry (Catalog # 16-266) to 500 µL of PBS in a microcentrifuge tube.
2. Gently rock the reaction mixture at 4°C for 1 hour.
3. Collect the agarose beads by pulsing (5 seconds in the microcentrifuge at 14,000 x g), and drain off the supernatant. Wash the beads 3 times with either ice-cold cell lysis buffer or PBS.
4. Dilute the cell lysate to roughly 1 µg/µL total cell protein with PBS.
5. Add 500 µg-1 mg cell lysate to the reaction mixture.
6. Gently rock the reaction mixture at 4°C for 1 hour.
7. Collect the agarose beads by pulsing (5 seconds in the microcentrifuge at 14,000 x g), and drain off the supernatant.
8. Wash the beads 3 times with either ice-cold cell lysis buffer or PBS.
9. Resuspend the agarose beads in 60 µL 2X Laemmli sample buffer.
10. Store the beads frozen for future analysis or boil the beads for 5 minutes.
11. Collect the beads after boiling using a microcentrifuge pulse.
12. Perform SDS-PAGE and western blot analysis on a sample of the supernatant fraction.

Phosphatase Treatment of Src-Containing Immunocomplexes

1. Follow steps 1 through 8 of the immunoprecipitation protocol.
2. Suspend the agarose beads in the appropriate phosphatase buffer containing either 6000 Units of YOP (Yersinia PTP) or 1,000 Units of Lambda Protein Phosphatase (Catalog # 14-405).
3. Gently rock the reaction mixture at 30°C for 1 hour.
4. Wash the beads 3 times with PBS.
5. Continue with step 9 of the immunoprecipitation protocol above.

RELATED PRODUCTS (specific)

cat #	description
16-248	■ Anti-phospho-Src (Tyr416), clone 9A6, Alexa Fluor® 488 conjugate
16-249	■ Anti-phospho-Src (Tyr416), clone 9A6, Alexa Fluor® 555 conjugate
07-909	■ anti-phospho-Src (Tyr418)
07-910	■ anti-phospho-Src (Tyr529)
05-857	■ Anti-phospho-Src family (Tyr416), clone 2N8
07-791	■ anti-Src (Tyr215)
16-186	■ Anti-Src, clone GD11, agarose
05-889	■ Anti-Src, clone N6L
05-772	■ Anti-Src, CT, clone NL19
17-426	■ Src Phospho-ELISA Assay Kit (Chemiluminescent)
17-468	■ STAR phospho-Src (Tyr418) ELISA Kit
17-467	■ STAR Src ELISA Kit
14-117	■ Src (p60c-src)
14-326	■ Src, active
12-349	■ Goat Anti-Mouse IgG, HRP conjugate

RELATED PRODUCTS (non-specific)

cat #	description
IPVH00010	■ Immobilon-P 26.5 cm x 3.75 m Roll PVDF 0.45 µm
IPFL00010	■ Immobilon-FL 26.5 cm x 3.75 m Roll PVDF 0.45 µm
IPVH07850	■ Immobilon-P 7 x 8.4 cm PVDF 0.45 mm (sheet) 50/pk
ISEQ00010	■ Immobilon-P SQ 26.5 cm x 3.75 m 1 roll PVDF 0.2 µm
ISEQ07850	■ Immobilon-P 7 x 8.4 cm PVDF 0.2 mm (sheet) 50/pk
IPFL07810	■ Immobilon-FL 7 x 8.4 cm PVDF 0.45 mm (sheet) 10/pk
WBKLS0100	■ Immobilon Western Chemilum HRP Substrate 100 mL
17-373	■ Spray & Glow™ ECL WB Detection System 1 ea
2060	■ Re-Blot Western Blot Recycling Kit
2500	■ Re-Blot Plus Western Blot Recycling Kit
B2080-175GM	■ Blot Quick Blocker Membrane Blocking Agent 175G

■ antibodies ■ Multiplex products ■ biotools ■ cell culture ■ enzymes ■ kits ■ proteins/peptides ■ siRNA/cDNA products

Please visit www.millipore.com for additional product information, test data and references

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