

Certificate of Analysis

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

(mouse monoclonal IgG₃)

Catalog # 05-677

Lot # 33447

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

Specificity: Recognizes phospho-Src, Mr 60kDa. The antibody does not crossreact with the non-phosphorylated 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.

Species Cross-reactivity: Human, mouse, and rat.

Formulation: 100µg of mouse monoclonal IgG₃ lyophilized from 50µl of PBS, 0.1% sodium azide, PEG, and sucrose.

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

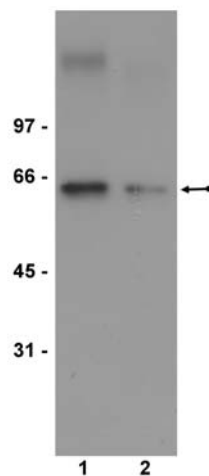
Storage and Stability: Lyophilized: stable for 1 year at -20°C from date of shipment. Rehydrated: Stable for 3 months at 4°C. Aliquot solution to store frozen and avoid repeated freeze thaw cycles.

**FOR IN VITRO RESEARCH USE ONLY
NOT FOR USE IN HUMANS OR ANIMALS**

Quality Control Testing

Src Autophosphorylation/Immunoblot Analysis: 0.5-2µg/ml of this lot 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.

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



Src Autophosphorylation/Immunoblot Analysis

Representative blot from a previous lot. Src, active (Catalog # 14-326) was treated with either Mg/Mn/ATP (lane 1) or λ-phosphatase (Catalog # 14-405), (lane 2). The reaction products were resolved by electrophoresis, transferred to nitrocellulose and probed with anti-phospho-Src (1µg/ml). Phosphorylated Src was visualized using a goat anti-mouse secondary antibody conjugated to HRP and a chemiluminescence detection system. Arrow indicates phospho-Src (~60kDa).

Application References:

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

Immunoprecipitation Protocol

1. Add **10µg of anti-phospho-Src (Tyr416), clone 9A6** and 60µl (30µl packed beads) of washed Protein G agarose bead slurry (Catalog # 16-266) to 500µl 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 1µg/µl total cell protein with PBS.
5. Add 500µg-1mg 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 60µl 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 immunoblot 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.

Src Autophosphorylation/Immunoblot Protocol

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 20µl (50ng 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 **0.5-2µg/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).