



cell signaling solutions

Certificate of Analysis

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

(mouse monoclonal IgG_{1κ})

Catalog # 05-677

Lot # 26419

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 IgG_{1κ} lyophilized from 53μl 2X PBS, 0.09% sodium azide, PEG, and sucrose. Reconstitute with 100μl H₂O to make a 1μg/μl solution.

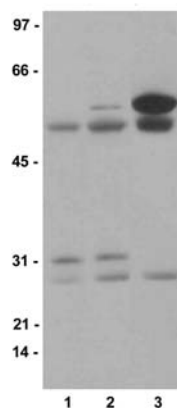
Storage and Stability: Lyophilized: stable for 1 year at -20°C from date of shipment. Rehydrated: Stable for 3 months at 4°C. Aliquot solution to store frozen and avoid repeated freeze thaw cycles.

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

Quality Control Testing

Src Autophosphorylation/Immunoblot Analysis: 1μg/ml of this lot strongly detected recombinant Src (Catalog # 14-326) after incubation with MnATP. Detection of recombinant Src before incubation MnATP was greatly reduced by treating the recombinant protein with Lambda Protein Phosphatase (Catalog # 14-405).

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.



Immunoprecipitation/Immunoblot Analysis

Representative blot from a previous lot. Src immunoprecipitated with anti-Src (Catalog # 05-184) was either treated (lane 1) or untreated (lanes 2 and 3) with the phosphotyrosine phosphatase YOP, then resolved by electrophoresis, transferred to nitrocellulose and probed with anti-phospho-Src (4μg/ml) lanes 1 and 2, or anti-Src (1μg/ml) lane 3. Proteins were visualized using a goat anti-mouse secondary antibody conjugated to HRP and a chemiluminescence detection system. Arrow indicates phospho-Src (~60kDa).

Immunoprecipitation Protocol

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

Immunoblot Protocol

1. Perform SDS-polyacrylamide gel electrophoresis (SDS-PAGE) on the immunoprecipitate from above or a cell lysate sample (cell lysis buffer: 50mM Tris-HCl, pH 7.4; 1% NP-40; 0.25% sodium deoxycholate; 150mM NaCl; 1mM EDTA; 1mM PMSF; 1 μ g/ml each aprotinin, leupeptin, pepstatin; 1mM Na₃VO₄, 1mM NaF) and transfer the proteins to nitrocellulose. Wash the blotted nitrocellulose twice with water.
2. Block the blotted nitrocellulose in freshly prepared 3% nonfat dry milk (Catalog # 20-200) in TBS with 0.05% Tween 20 (TBST-MLK) for 20 minutes at room temperature with constant agitation.
3. Incubate the nitrocellulose with **4 μ g/ml of anti-phospho-Src (Tyr416), clone 9A6**, diluted in freshly prepared TBST-MLK overnight with agitation at 4°C.
4. Wash the nitrocellulose twice with water.
5. Incubate the nitrocellulose in the secondary reagent of choice (a goat anti-mouse HRP conjugated IgG, Catalog # 12-349, 1:2000 dilution was used) in TBST-MLK for 1.5 hours with agitation at room temperature.
6. Wash the nitrocellulose twice with water.
7. Wash the nitrocellulose in TBS-0.05% Tween 20 for 3-5 minutes.
8. Rinse the nitrocellulose in 4-5 changes of water.
9. Use detection method of choice (enhanced chemiluminescence was used).

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.