

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

(mouse monoclonal IgG_{1κ})

Catalog # 05-677

Lot # 22412

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 Y416 of avian Src.

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

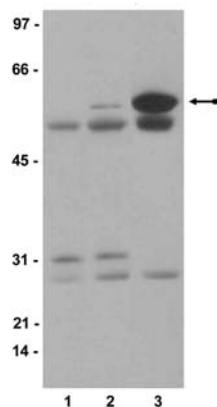
Formulation: 100µg of mouse monoclonal IgG_{1κ} lyophilized from 1ml 2X PBS, 0.1% sodium azide, PEG, and sucrose. Reconstitute with 1ml H₂O.

Storage and Stability: Lyophilized: stable for 1 year at -20°C from date of shipment. Rehydrated: Stable for 3 months at 4°C.

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

Quality Control Testing

Immunoblot Analysis: 4µg/ml of this lot detected phospho-Src immunoprecipitated from 3T3 cell lysates with 10µg of anti-Src (Catalog # 05-184) but not when the immunocomplex was treated with YOP (Yersinia PTP, Catalog # 14-229).



Immunoblot Analysis

Src immunoprecipitated with anti-Src (Catalog # 05-184) was either treated (lane 1) or untreated (lanes 2 and 3) with the phosphotyrosine phosphatase YOP (Catalog # 14-229), then resolved by electrophoresis, transferred to nitrocellulose and probed with anti-phospho-Src (4µg/ml) lanes 1 and 2, or anti-Src (Catalog # 05-184, 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. Dilute the cell lysate before beginning the immunoprecipitation to roughly $1\mu\text{g}/\mu\text{l}$ total cell protein in a microcentrifuge tube with PBS.
2. Add **10 μg of anti-Src** (Catalog # 05-184) to 1mg 3T3 cell lysate.
3. Gently rock the reaction mixture at 4°C for 1 hour.
4. Capture the immunocomplex by adding $60\mu\text{l}$ ($30\mu\text{l}$ packed beads) of washed Protein G agarose bead slurry (Catalog # 16-266).
5. Gently rock the reaction mixture at 4°C for 1 hour.
6. Collect the agarose beads by pulsing (5 seconds in the microcentrifuge at $14,000 \times g$), and drain off the supernatant. Wash the beads 2 times with either ice-cold cell lysis buffer or PBS.
7. Suspend the agarose beads in $50\mu\text{l}$ 2X Laemmli sample buffer.
8. Store the beads frozen for future analysis or boil the beads for 5 minutes.
9. Collect the beads after boiling using a microcentrifuge pulse.
10. Perform SDS-PAGE and immunoblot analysis on a sample of the supernatant fraction.

Phosphatase Treatment of Src-containing immunecomplexes

1. Follow steps 1 through 6 of the above immunoprecipitation protocol.
2. Suspend the agarose beads in 6000 unit of YOP (Yersinia PTP, Catalog # 14-229).
3. Gently rock the reaction mixture at 30°C for 1 hour.
4. Wash the beads 2 times with PBS.
5. Continue with step 7 of the above immunoprecipitation protocol.

Immunoblot Protocol

1. Perform SDS-polyacrylamide gel electrophoresis (SDS-PAGE) on a sample (cell lysis buffer: 50mM Tris-HCl, pH 7.4; 1% NP-40; 0.25% sodium deoxycholate; 150mM NaCl; 1mM EGTA; 1mM PMSF; $1\mu\text{g}/\text{ml}$ each aprotinin, leupeptin, pepstatin; 1mM Na_3VO_4 ; 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 $\mu\text{g}/\text{ml}$ of anti-phospho Src**, 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 from Amersham, Catalog # NA931A, 1:5000 dilution was used) in TBST-MLK for 1 hour at room temperature with agitation.
6. Wash the nitrocellulose twice with water.
7. Wash the nitrocellulose in TBS-MLK for 3-5 minutes.
8. Rinse the nitrocellulose in 4-5 changes of water.
9. Use detection method of choice (enhanced chemiluminescence was used).