



cell signaling solutions

## Certificate of Analysis

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

(mouse monoclonal IgG<sub>1κ</sub>)

Catalog # 05-677

Lot # 24176

**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<sub>1κ</sub> lyophilized from 66µl 2X PBS, 0.09% sodium azide, PEG, and sucrose. Reconstitute with 1ml H<sub>2</sub>O.

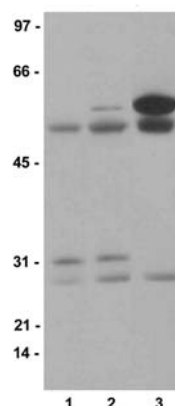
**Storage and Stability:** Lyophilized: stable for 1 year at -20°C from date of shipment. Rehydrated: Stable for 3 months at 4°C.

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

### Quality Control Testing

**Immunoblot Analysis:** 4µg/ml of this lot detected recombinant Src (Catalog # 14-326), but not after phosphatase treatment with YOP (Yersinia PTP, Catalog # 14-229).

**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 (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 (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 $\mu\text{g}$  of anti-Src** (Catalog # 05-184) to 1mg PDGF stimulated 3T3 cell lysate.
3. Gently rock the reaction mixture at  $4^{\circ}\text{C}$  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  $4^{\circ}\text{C}$  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  $60\mu\text{l}$  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  $30^{\circ}\text{C}$  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  $\text{Na}_3\text{VO}_4$ ; 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  $4^{\circ}\text{C}$ .
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).