

## Certificate of Analysis

**Anti-phospho-Src (Tyr416), clone 9A6**  
(mouse monoclonal IgG<sub>1κ</sub>)  
Catalog # 05-677  
Lot # 31941

**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 50μl of PBS, 0.1% sodium azide, PEG, and sucrose.

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

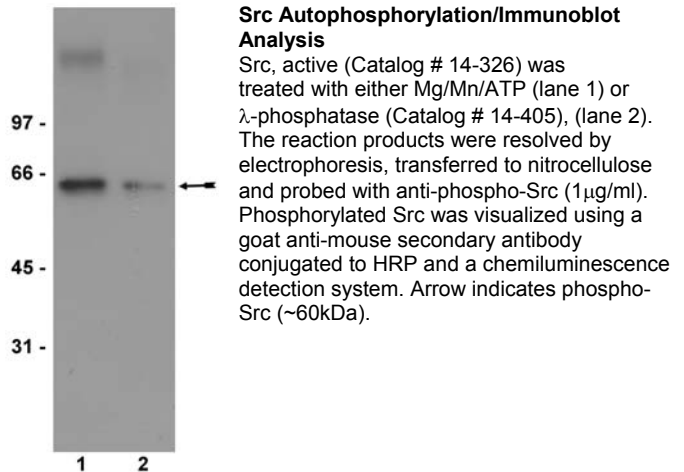
**Storage and Stability:** Lyophilized: stable for 1 year at -20°C from date of shipment. Rehydrated: Stable for 3 months at 4°C. Aliquot solution to store frozen and avoid repeated freeze thaw cycles.

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

### Quality Control Testing

**Src Autophosphorylation/Immunoblot Analysis:** 1μg/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.



### Application References:

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

### Immunoprecipitation Protocol

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

### 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  $\lambda$ -phosphatase treatment and one minus (-) for no  $\lambda$ -phosphatase treatment.
2. Prepare each tube as described in the following table:

	$\lambda$ -phosphatase (-)	$\lambda$ -phosphatase (+)
Src Manganese/ATP Cocktail (Catalog # 20-110)	12.5 $\mu$ l	-
Src Kinase Reaction Buffer (Catalog # 20-121)	12.5 $\mu$ l	12.5 $\mu$ l
Src, active (Catalog # 14-326)	5 $\mu$ l (500ng)	5 $\mu$ l
sterile, distilled water	20 $\mu$ l	21.5 $\mu$ l
DTT (Catalog # 20-265)	-	1 $\mu$ l
Lambda Phosphatase (Catalog # 14-405)	-	10 $\mu$ l (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 $\mu$ l (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 30-45 minutes at room temperature with constant agitation.
9. Incubate the nitrocellulose with **1 $\mu$ g/ml of anti-phospho-Src (Tyr416), clone 9A6**, diluted in freshly prepared TBST-MLK overnight with agitation at 4°C.
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, 1:4000 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).