

Certificate of Analysis

Anti-phospho-Src (Tyr418)
 (Affinity purified Rabbit polyclonal IgG)
 Catalog # 07-909
 Lot #

Immunogen: Peptide corresponding to amino acid region encompassing the human, mouse, rat, chicken, and frog phospho-Src (Tyr418)

Specificity: Recognizes phosphorylated Src (Tyr418)

Species Cross-reactivity: Human, Mouse and Chicken tested. Predicted to cross-react with Rat and Frog (100% homologous) based on sequence homology.

Formulation: 100 µL of affinity purified rabbit polyclonal IgG in 50% storage buffer (PBS (without Mg²⁺ and Ca²⁺), pH 7.3 containing 1.0 mg/mL BSA (IgG, protease free) and 0.05% sodium azide) and 50% glycerol.

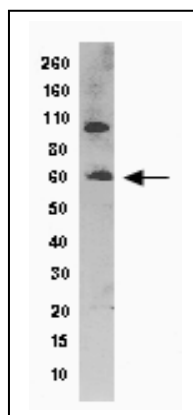
Storage and Stability: Stable for 2 years at -20°C from date of shipment.

Handling Recommendations: Upon first thaw, and prior to removing the cap, centrifuge the vial and gently mix the solution. Aliquot into microcentrifuge tubes and store at -20°C. **Avoid repeated freeze/thaw cycles, which may damage IgG and affect product performance.** Note: Variability in freezer temperatures below -20°C may cause glycerol-containing solutions to become frozen during storage

FOR RESEARCH USE ONLY; NOT FOR USE IN DIAGNOSTIC PROCEDURES. NOT FOR HUMAN OR ANIMAL CONSUMPTION

Quality Control Testing

Immunoblot Analysis: 1:500 – 1:2000 dilutions of this lot detected phospho-Src (Tyr418) in RIPA lysate of E16 Rat Heart.



Immunoblot
 REPRESENTATIVE LOT DATA
 Lysates from E16 Rat Heart cells were resolved by electrophoresis, transferred to PVDF, and probed with anti-phospho-Src(Tyr418) (1/500). Proteins were visualized via HRP and chemiluminescent detection. Arrow indicates phospho-Src.

General References:

1. Ishizawar, R. and Parsons, S.J., *Cancer Cell* **6**: 209-214, 2004.
2. Lowell, C.A., *Mol. Immunol.* **41**: 631-643, 2004.
3. Roskoski, R. Jr., *Biochem. Biophys. Res. Commun.* **324**: 1155-1164, 2004.
4. Tsygankov, A.Y. and Shore, S.K., *Curr. Pharm. Des.* **10**: 1745-1756, 2004.

Immunoblot Protocol

1. Perform SDS-polyacrylamide gel electrophoresis (SDS-PAGE) on a E16 Rat Heart Lysate (cell lysis buffer: 50mM Tris-HCl, pH 7.4; 1% NP-40; 0.25% sodium deoxycholate; 150mM NaCl; 1mM EDTA; 1mM PMSF; 1µg/ml each aprotinin, leupeptin, pepstatin; 1mM Na₃VO₄, 1mM NaF) and transfer the proteins to PVDF. Wash the blotted PVDF twice with water.
2. Block the blotted PVDF in freshly prepared 3% nonfat dry milk (Catalog # 20-200) in PBS with 0.05% Tween[®]-20 (PBS-MLK) for 20 minutes at room temperature with constant agitation.
3. Incubate the PVDF with 1:500 - 1:2000 dilution of **anti-Src (Tyr418)**, diluted in freshly prepared PBS-MLK overnight with agitation at 4°C.
4. Wash the PVDF twice with water.
5. Incubate the PVDF in the secondary reagent of choice (a goat anti-rabbit HRP conjugated IgG, (Catalog # 12-348, 1:5000 dilution was used) in PBS-MLK for 1.5 hours with agitation at room temperature.
6. Wash the PVDF twice with water.
7. Wash the PVDF in PBS-0.05% Tween[®]-20 for 3-5 minutes.
8. Rinse the PVDF in 4-5 changes of water.
9. Use detection method of choice (enhanced chemiluminescence was used).

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