



cell signaling solutions

## Certificate of Analysis

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### Anti-Lck

(rabbit polyclonal IgG)

Catalog # 06-583

Lot # 25168

**Immunogen:** GST fusion protein corresponding to amino acids 1-58 of human Lck.

**Specificity:** recognizes Lck, Mr 56kDa; reactivity with other Src family members not determined.

**Cross-reactivity:** Mouse, human and bovine.

**Formulation:** 200µg of protein A purified rabbit IgG in 200µl of 0.1M Tris-glycine, pH 7.4, 0.15M NaCl with 0.05% sodium azide. Frozen solution.

**Storage and Stability:** Stable for 2 years at -20°C from date of shipment. Aliquot to avoid repeated freezing and thawing. For maximum recovery of product, centrifuge the vial after thawing and prior to removing the cap.

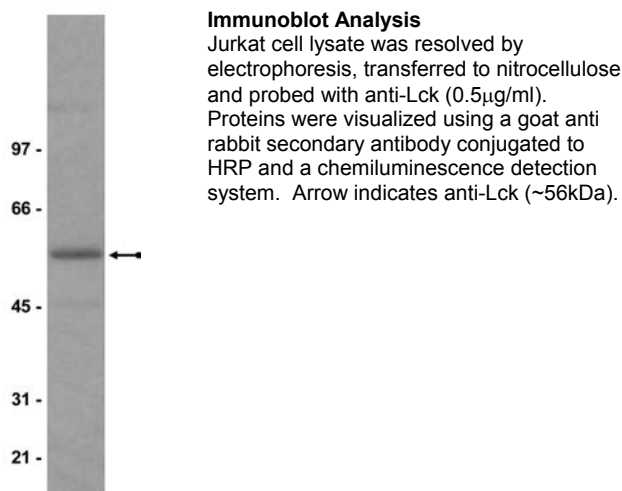
**FOR RESEARCH USE ONLY  
NOT FOR USE IN HUMANS**

### Quality Control Testing

**Immunoblot Analysis:** 0.5-2µg/ml of this lot detected Lck in RIPA lysates from Jurkat cells.

**Included Positive Antigen Control:** Catalog # 12-303, Jurkat cell lysate. **Add 2.5µl of 2-mercaptoethanol/100µl of lysate and boil for 5 minutes to reduce the preparation.** Load 20µg of reduced lysate per lane for minigels.

**Immunoprecipitation:** 4µg of this lot immunoprecipitated Lck from 1mg of RIPA lysates from Jurkat cells.



### General References:

Rouer, E., *et al.*, *Cell Growth and Differentiation* **5**: 659-666, 1994.  
Marth, J. D., *et al.*, *EMBO J.* **6**: 2727-2734, 1987.  
Shin, S., and D.L. Steffen, *Oncogene* **8**: 141-149, 1993.

### Application References:

Boytim, Michelle L., *et al.*, *J. Clin. Invest.* **105**: 1447-1453, 2000.  
Musch, M.W., *et al.*, *J. Biol. Chem.* **274**: 7923-7928, 1999.

### Immunoblot Protocol

1. Perform SDS-polyacrylamide gel electrophoresis (SDS-PAGE) on a cell lysate sample (cell lysis buffer: 50mM Tris-HCl, pH7.4; 1% NP-40; 0.25% sodium deoxycholate; 150mM NaCl; 1mM EGTA; 1mM PMSF; 1 $\mu$ g/ml each aprotinin, leupeptin, pepstatin; 1mM Na<sub>3</sub>VO<sub>4</sub>; 1mM NaF) and transfer the proteins to nitrocellulose. Wash the blotted nitrocellulose twice with water.
2. Block the blotted nitrocellulose in freshly prepared PBS containing 3% nonfat dry milk (Catalog # 20-200), (PBS-MLK) for 20-30 minutes at room temperature with constant agitation.
3. Incubate the nitrocellulose with **0.5-2 $\mu$ g/ml of anti-Lck**, diluted in freshly prepared PBS-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-rabbit** HRP conjugated IgG, Catalog # 12-348, 1:5000 dilution was used) in PBS-MLK for 1.5 hours at room temperature with agitation.
6. Wash the nitrocellulose with water twice.
7. Wash the nitrocellulose in PBS-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).

### Immunoprecipitation Protocol

1. Add **4 $\mu$ g of anti-Lck** and 60 $\mu$ l (30 $\mu$ l packed beads) of washed Protein A agarose bead slurry (Catalog # 16-125) 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 2 hours.
7. 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.
8. Resuspend the agarose beads in 60 $\mu$ l 2X Laemmli sample buffer.
9. Store the beads frozen for future analysis or boil the beads for 5 minutes.
10. Collect the beads after boiling using a microcentrifuge pulse.
11. Perform SDS-PAGE and immunoblot analysis on a sample of the supernatant fraction.