

**Anti-Lck**  
(rabbit polyclonal IgG)  
Catalog # 06-583  
Lot # 18782

**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.

**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.

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

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

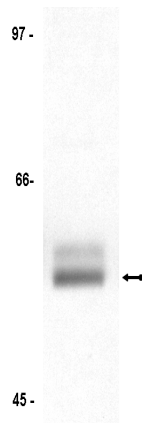
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**Quality Control Testing**

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

**Included Positive Antigen Control:** Jurkat cell lysate, Catalog # 12-303. Use 20µg per lane for minigels.

**Immunoprecipitation:** 4µg of this lot immunoprecipitated Lck from 1mg of Jurkat RIPA lysate as confirmed by subsequent immunoblot using monoclonal anti-Lck (Catalog # 05-435).



**Immunoblot Analysis**

Jurkat cell lysate was resolved by electrophoresis, transferred to nitrocellulose and probed with anti-Lck (1µg/ml). Proteins were visualized using a goat anti rabbit secondary antibody conjugated to HRP and a chemiluminescence detection system. Arrow indicates anti-Lck.

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**Background:** The lymphocyte-specific protein tyrosine kinase Lck is a member of the src family of nonreceptor tyrosine kinases. Abundant expression of Lck in normal and transformed T-cells, as well as in lymphoid tissue, suggests that Lck plays a role in lymphoid cell growth and differentiation. Furthermore, Lck is associated with interleukin receptors and is involved during Epstein-Barr virus infection leading to lymphocyte immortalization.

**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.

### 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 (PBS-MLK) for 20 minutes at 20-25°C with constant agitation.
3. Incubate the nitrocellulose with **0.5-2ng/ml of a-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, 1:3000 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. Dilute the cell lysate before beginning the immunoprecipitation to roughly 1 $\mu$ g/ $\mu$ l total cell protein in a microcentrifuge tube with PBS.
2. Add **4ng of a-Lck** to 500 $\mu$ g-1mg cell lysate.
3. Gently rock the reaction mixture at 4°C overnight.
4. Capture the immunocomplex by adding 100 $\mu$ l of washed Protein A agarose bead slurry (50 $\mu$ l packed beads).
5. Gently rock the reaction mixture at 4°C for 2 hours.
6. 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.
7. Resuspend the agarose beads in 50 $\mu$ l 2X Laemmli sample buffer.
8. The agarose beads can either be frozen for later use or suspended in Laemmli sample buffer and boiled for 5 minutes. Collect the beads by a microcentrifuge pulse. SDS-PAGE and subsequent immunoblot analysis can be performed on a sample of the supernatant.