

Anti-Human Lck

(rabbit polyclonal IgG)

Catalog # 06-583

Lot # 15799

Immunogen: GST fusion protein corresponding to aa 1-58 of human Lck.

Specificity: recognizes Lck (56kDa); reactivity with other Src family members not determined.

Cross-reactivity: Mouse and bovine.

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

Physical form: **200mg** of protein A purified anti-human Lck in **200ml** of 1M Tris-glycine, pH 7.5, and 0.05% sodium azide. Frozen solution.

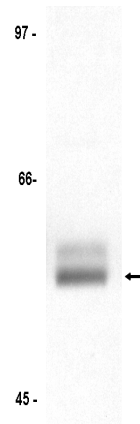
**FOR RESEARCH USE ONLY
NOT FOR USE IN HUMANS**

Quality Control Testing

Immunoblot Analysis: 1µg/ml of this lot detected Lck in RIPA lysates from Jurkat cells.

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

Immunoprecipitation: 5µg of this lot immunoprecipitated Lck from 1mg of Jurkat RIPA lysate.



Immunoblot Analysis

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

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 Journal **6**: 2727-2734, 1987.
Shin, S., and Steffen, D.L., 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; 1µg/ml each aprotinin, leupeptin, pepstatin; 1mM Na₃VO₄; 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 **1ng/ml of a-Human 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 1µg/µl total cell protein in a microcentrifuge tube with PBS.
2. Add **5ng of a-Human Lck** to 500µg-1mg cell lysate.
3. Gently rock the reaction mixture at 4°C overnight.
4. Capture the immunocomplex by adding 100µl of washed Protein A agarose bead slurry (50µl 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 50µl 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.