

**Anti-Lck, Clone 3A5**  
(mouse monoclonal IgG<sub>2b/k</sub>)  
Catalog # 05-435  
Lot # 19367

**Immunogen:** GST-fusion protein corresponding to amino acids 1-225 of murine Lck.

**Specificity:** Recognizes and is specific for Lck, Mr ~56kDa. Clone 3A5.

**Species Cross-reactivity:** Mouse, human and rat. Other species cross-reactivity not tested.

**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 original vial after thawing and prior to removing the cap.

**Note:** Add sodium azide to 0.05% for long term storage at 4°C.

**Formulation:** 100ng of protein A purified IgG<sub>2b/k</sub> in 100µl of 10mM PBS, pH 7.4. Frozen solution.

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

**Quality Control Testing**

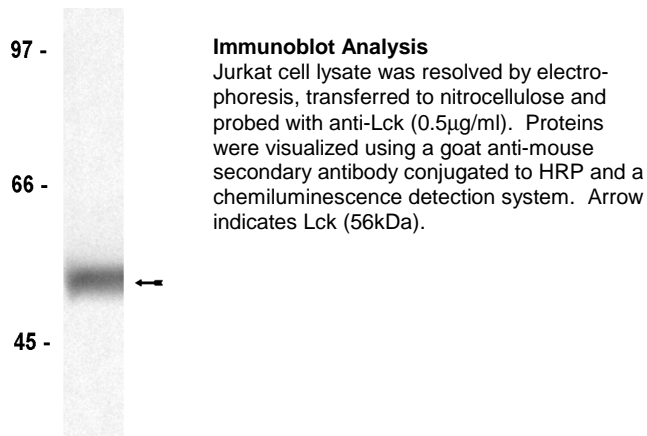
**Immunoblot Analysis:** 0.5-2µg/ml of this lot detected Lck in RIPA lysates from Jurkat cells.

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

**Immunoprecipitation:** 3µg of a previous lot immunoprecipitated Lck from 1mg of Jurkat RIPA lysate.

**Additional Research Applications**

**Immunoprecipitation Kinase Assay:** Reported to immunoprecipitate enzymatically active Lck as determined by immune complex kinase assays.



**General References:**

Omri, B., *et al.*, *J. Neurochem.* **67**: 1360-1364, 1996.  
Ley, S.C., *et al.*, *J. Cell. Biol.* **125**: 639-649, 1994.

### 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 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-mouse** HRP conjugated IgG, 1:4000 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 **3ng of anti-Lck** to 500 $\mu$ g-1mg cell lysate.
3. Gently rock the reaction mixture at 4°C overnight.
4. Capture the immunocomplex by adding 50 $\mu$ l of washed Protein A agarose bead slurry (25 $\mu$ 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.