



cell signaling solutions

## Certificate of Analysis

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**Anti-Lck, clone 3A5**  
(mouse monoclonal IgG<sub>2bk</sub>)  
Catalog # 05-435  
Lot # 27960

**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.

**Formulation:** 100µg of protein A purified IgG<sub>2bk</sub> in 100µl of 10mM PBS, pH 7.4 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 original vial after thawing and prior to removing the cap.

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

### Quality Control Testing

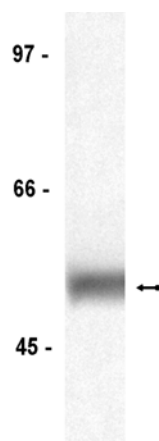
**Immunoblot Analysis:** 0.5-2µg/ml of this lot detected Lck in RIPA lysates from Jurkat cells.

**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:** 3µg of this 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.



#### Immunoblot Analysis

Representative blot from a previous lot. Jurkat cell lysate was resolved by electrophoresis, transferred to nitrocellulose and probed with anti-Lck (0.5µg/ml). Proteins were visualized using a goat anti-mouse secondary antibody conjugated to HRP and a chemiluminescence detection system. Arrow indicates Lck (~56kDa).

### General References:

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

### 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 EDTA; 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 3% nonfat dry milk (Catalog # 20-200) in PBS (PBS-MLK) for 20 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-mouse** HRP conjugated IgG, Catalog # 12-349, 1:1000 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 **3 $\mu$ g 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 (25 $\mu$ l packed beads) of washed Protein A agarose bead slurry (Catalog # 16-125).
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 60 $\mu$ l 2X Laemmli sample buffer.
8. Store the beads frozen for future analysis or boil the beads for 5 minutes.
9. Collect the beads after boiling using a microcentrifuge pulse.
10. Perform SDS-PAGE and immunoblot analysis on a sample of the supernatant fraction.