

Certificate of Analysis

Anti-Lck, clone 3A5
(mouse monoclonal IgG_{2bκ})
Catalog # 05-435
Lot # 22708

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.

Formulation: 100µg of protein A purified IgG_{2bκ} in 100µl of 10mM PBS, pH 7.4 with 0.05% sodium azide. Frozen solution.

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

Quality Control Testing

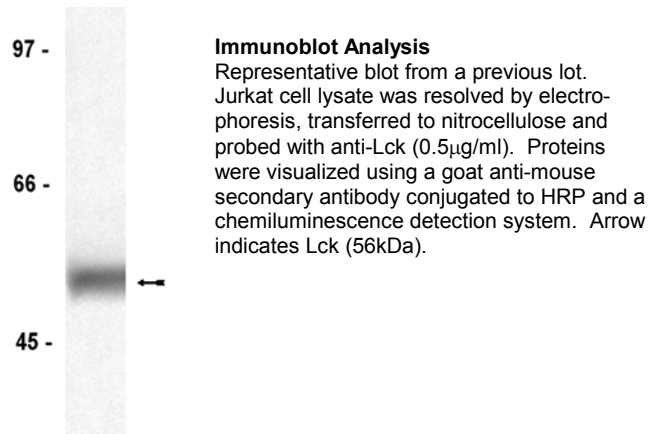
Immunoblot Analysis: 0.5-2µg/ml of this lot detected Lck in RIPA lysates from Jurkat cells.

Included Positive Antigen Control: Catalog # 12-303, Jurkat cell lysate. **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 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 μ g/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 3% nonfat dry milk (Catalog # 20-200) in PBS (PBS-MLK) for 20 minutes at 20-25°C with constant agitation.
3. Incubate the nitrocellulose with **0.5-2 μ 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, 1:2000 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 μ g/ μ l total cell protein in a microcentrifuge tube with PBS.
2. Add **3 μ g of anti-Lck** to 500 μ g-1mg cell lysate.
3. Gently rock the reaction mixture at 4°C overnight.
4. Capture the immunocomplex by adding 50 μ l (25 μ 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 50 μ 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.