

Certificate of Analysis

Lck, unactive
 (recombinant protein expressed in Sf21 cells)
 Catalog # 14-708
 Lot # 30448

Product Description: N-terminal 6His-tagged, recombinant full-length human Lck expressed in Sf21 insect cells. Purified using Ni²⁺/NTA agarose. The purified Lck was unactivated through dephosphorylation with a phosphatase, which was subsequently removed by chromatography. Purity 95% by SDS-PAGE and Coomassie blue staining. MW = 59kDa.

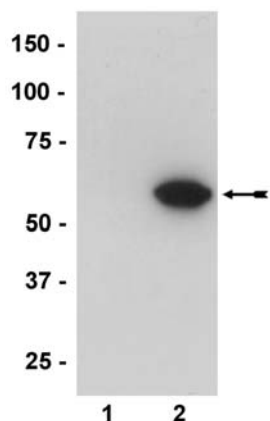
Specific Activity (lot # 30448): As provided, this lot demonstrated <1% of maximum activity.

Formulation: 5µg recombinant protein in 50µl of 50mM Tris-HCl, pH 7.5, 0.15M NaCl, 0.1mM EGTA, 0.03% Brij-35, 5% glycerol, 0.2mM PMSF, 0.1% β-mercaptoethanol. Frozen solution.

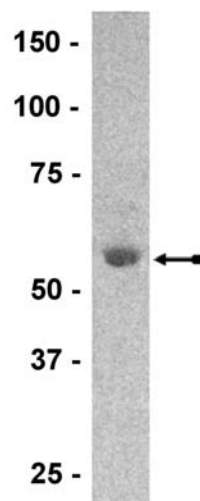
Storage and Stability: Stable for 6 months at -70°C from date of shipment. For maximum recovery of product, centrifuge the original vial after thawing and prior to removing the cap. Aliquot to avoid repeated thawing and freezing.

**FOR IN VITRO RESEARCH USE ONLY
 NOT FOR USE IN HUMANS OR ANIMALS**

Quality Control Testing and Research Applications



Immunoblot Analysis
 Lck, unactive (lane 1) and Lck, active (Catalog # 14-442, lane 2) were resolved by electrophoresis and probed with anti-phospho-Src (Tyr416), clone 9A6 (Catalog # 05-677, which recognizes phosphorylated members of the Src family, including Lck). Proteins were visualized using a goat anti-mouse secondary antibody conjugated to HRP and a chemiluminescence detection system. Arrow indicates phosphorylated Lck (~59kDa).



SDS-PAGE and Coomassie Stain
 Representative gel from this lot. Purity was assessed by SDS-PAGE and Coomassie blue staining using 1µg of Lck, unactive. Arrow indicates Lck, unactive (~59kDa).

Lck Kinase Assay: 100ng of this lot of enzyme phosphorylated 250µM Src substrate peptide (Catalog # 12-140) in the assay described on page two.

Lck, unactive	Lck, active (14-442)	Substrate	Mean CPM	Comment
none	none	250µM	6,188	Substrate background
100ng	none	none	6,329	Enzyme background
none	5ng	250µM	17,552	Lck, active activity + background
100ng	none	250µM	7,760	Lck, unactive activity + background
95ng	5ng	250µM	376,436	Lck, reactivated activity + background

Kinase Assay Procedure

Stock Solutions:

1. Assay Buffer: 200mM Tris-HCl pH 7.5, 0.4mM EGTA, 0.4mM sodium orthovanadate.
2. Lck Formulation Buffer: 50mM Tris-HCl, pH 7.5, 0.15M NaCl, 0.1mM EGTA, 0.03% Brij-35, 5% glycerol, 0.2mM PMSF, 0.1% β -mercaptoethanol.
3. Lck, reactivated: Prepare 100 μ l of reactivated Lck by adding 2 μ l of Magnesium/ATP cocktail to 10 μ l of a mixture containing 950ng of Lck, inactive (Catalog # 14-708) and 50ng of Lck, active (Catalog # 14-442). Incubate at 30°C for 30 minutes, then dilute with 88 μ l of Lck formulation buffer. Use 10 μ l per assay point.
4. Src Substrate Peptide (Catalog # 12-140): Prepare a 2.5mM stock with sterile, distilled water. Use 4 μ l per assay point for a final concentration of 250 μ M per assay.
5. Magnesium/ATP Cocktail (Catalog # 20-113): 500 μ M cold ATP and 75mM magnesium chloride in 20mM MOPS, pH 7.2, 25mM β -glycero-phosphate, 5mM EGTA, 1mM Na₃VO₄, 1mM dithiothreitol.
6. [γ -³²P]ATP: Stock 1mCi/100 μ l (3000Ci/mmol, obtained from PerkinElmer, Catalog # BLU002A). Make 10 μ l aliquots (100 μ Ci/vial). Before starting the assay, dilute the Magnesium/ATP Cocktail 1:1 with sterile, distilled water. Then dilute an aliquot of [γ -³²P]ATP to 1 μ Ci/ μ l with 90 μ l of the diluted Magnesium/ATP Cocktail. Use 10 μ l per assay point for a final ATP concentration of 100 μ M per assay point.

Assay Protocol:

1. Add 10 μ l of Assay Buffer per assay.
2. Add **10 μ l (100ng) of reactivated Lck.**
3. Add 4 μ l Src Substrate peptide (250 μ M).
4. Add 6 μ l distilled water.
5. Add 10 μ l of the diluted [γ -³²P]ATP solution.
6. Incubate for 10 minutes at 30°C.
7. Transfer a 20 μ l aliquot onto the center of a 2cm x 2cm P81 paper.
8. Wash assay squares five times with 0.75% phosphoric acid for 5 minutes per wash.
9. Wash assay squares once with acetone for 5 minutes.
10. Transfer assay squares to a scintillation vial and add 5ml scintillation cocktail.
11. Read in scintillation counter. Compare CPM of enzyme samples to CPM of control samples that contain no substrate (background control).

Technical Note: Allow the radiolabeled substrate to bind to the filter paper for 30 seconds before immersing the paper into a 50ml conical tube containing 40ml 0.75% phosphoric acid. Gently shake the assay squares for 5 minutes on a rotator. Discard the wash in a liquid radioisotope waste container, (dispose of per institutional regulations) and repeat the wash step twice. Wash the squares in 20ml of acetone for 5 minutes. Drain and add scintillation cocktail.

Lck Sequence Information

Protein Human Lck
Tag N-terminal 6His (H2-H7)
Native sequence G9 of recombinant protein (bold, underlined) is equivalent to G1 of human Lck.
Accession number Swiss-Prot P06239

Recombinant Lck amino acid sequence:

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1  MHHHHHHMGC GCSHPEDDW MENIDVCENC HYPIVPLDGK GTLLIRNGSE VRDPLVITYEG
61  SNPPASPLQD NLVIALHSYE PSHDGLGFE KGEQLRILEQ SGEWWKAQSL TTGQEGFIPF
121 NFAKANSLE PEPWFFKNLS RKDAERQLLA PGNTHGSFLI RESESTAGSF SLSVRDFDQN
181 QGEVVKHYKI RNLDNGGFYI SPRITFPGLH ELVRHYTNAS DGLCTRLSRP CQTQKPQKPW
241 WEDEWEVPRE TLKLVERLGA GQFGEVVMGY YNGHTKVAVK SLKQGSMSPD AFLAEANLMK
301 QLQHQRQLVRL YAVVTQEPIY IITEYMENGS LVDFLKTSPG IKLTINKLLD MAAQIAEGMA
361 FIEERNYIHR DLRAANILVS DTLSCKIADF GLARLIEDNE YTAREGAKFP IKWTAPEAIN
421 YGTFTIKSDV WSFGILLTEI VTHGRIPYPG MTNPEVIQNL ERGYRMVRPD NCPEELYQLM
481 RLCWKERPED RPTFDYLRSV LEDFFTATEG QYQPOP
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Recombinant Lck nucleotide sequence:

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1  atgcatcacc atcaccatca tatgggctgt ggctgcagct cacacccgga agatgactgg
61  atggaaaaca tcgatgtgtg tgagaactgc cattatccca tagtcccact ggatggcaag
121 ggcacgctgc tcatccgaaa tggctctgag gtgcgggacc cactggttac ctacgaaggc
181 tccaatccgc cggcttcccc actgcaagac aacctgggta tcgctctgca cagctatgag
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