

Certificate of Analysis

Lck, active

(recombinant enzyme expressed in Sf21 insect cells)

Catalog # 14-442

Lot # 30945U

Product Description: N-terminal 6His-tagged, recombinant full-length human Lck expressed in Sf21 insect cells. Purified using Ni²⁺/NTA agarose. Purity 85% by SDS-PAGE and Coomassie blue staining. MW = 59kDa.

Specific Activity (lot 30945U): 375U/mg, where one unit of Lck activity is defined as 1nmol phosphate incorporated into 250µM cdc 2 peptide per minute at 30 °C with a final ATP concentration of 100µM.

Formulation: 10µg of enzyme in 5.2µl of 50mM Tris/HCl, pH7.5, 0.1mM EGTA, 150mM NaCl, 1mM benzamidine, 0.03% Brij-35, 0.2mM PMSF, 0.1% 2-mercaptoethanol, 5% glycerol. Frozen solution.

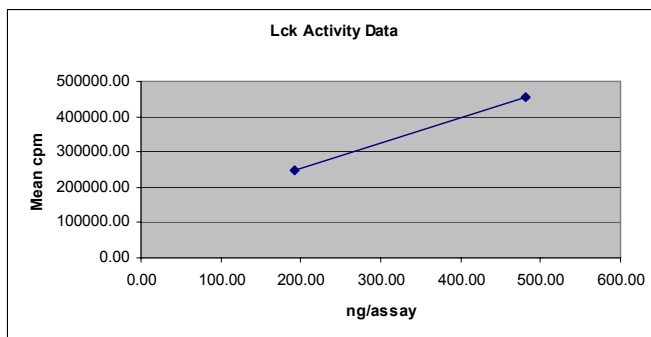
Storage and Stability: Stable for 1 year at -70°C from date of shipment. For maximum recovery of product, centrifuge original vial prior to removing the cap.

Handling Recommendations: Rapidly thaw the vial under cold water and immediately place on ice. Aliquot unused material into pre-chilled microcentrifuge tubes and immediately snap-freeze the vials in liquid nitrogen prior to re-storage at -70°C.

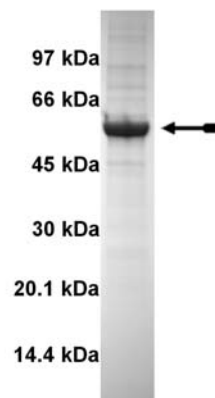
**FOR IN VITRO RESEARCH USE ONLY
NOT FOR USE IN HUMANS OR ANIMALS**

Quality Control Testing

Kinase Assay: 192 - 480ng of this lot of enzyme phosphorylated 250µM cdc2 peptide in the assay described on page two. Assay background was subtracted from the actual counts to yield the results shown below.



MS Tryptic Fingerprint: Confirmed identity as Lck with 61% amino acid coverage of the translated sequence listed on page 3.



SDS-PAGE and Coomassie Stain: Representative gel from this lot. Purity was assessed by SDS-PAGE and Coomassie blue staining using 3µg of Lck, active.

Kinase Assay Protocol

Stock Solutions:

1. **4 x Reaction Buffer:** 200mM Tris/HCl pH7.5, 0.4mM EGTA, 0.4mM Na₃VO₄.
2. **cdc2 Substrate peptide:** Use a final concentration of 250μM. Make a 2.5mM stock. Add 2.5μl of stock solution.
3. **Lck:** Dilute with 20mM MOPS/NaOH pH7.0, 1mM EDTA, 5% glycerol, 0.01% Brij 35, 0.1% 2-mercaptoethanol, 1mg/ml BSA. Use 192 - 480ng per assay point.
4. **[γ-³³P]ATP:** 2.5 x magnesium acetate/[γ-³³P]ATP cocktail: 25mM MgAc and 0.25mM ATP to which is added [γ-³³P]ATP (specific activity approximately 500 - 800cpm/pmol as required).

Assay Procedure (96 well plate format):

1. Add 6.25μl of 4 x reaction buffer per assay to wells.
2. Add 2.5μl of substrate peptide.
3. Add **2.5μl (192 – 480ng) Lck, active**.
4. Add 3.75μl of dH₂O.
5. Add 10μl of diluted [γ-³³P]ATP mixture.
6. Incubate for 10 minutes at 30°C.
7. Stop the reaction by adding 5μl of 3% phosphoric acid.
8. Transfer a 10μl aliquot onto the appropriate area of a **P30 Filtermat**.
9. Wash the filtermat twice for 5 minutes with 75mM phosphoric acid.
10. Wash the filtermat once for 2 minutes with methanol.
11. Transfer the filtermat to a sealable plastic bag and add 4ml of scintillation cocktail.
12. Read in a scintillation counter. Compare CPM of enzyme samples to CPM of control samples that contain all assay components and 1μl of 30% phosphoric acid (background CPM).

Lck Sequence Information

Protein Human Lck.
Tags N-terminal 6His tag.
Native sequence G9 of the recombinant protein 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 PSHDGD LGFE KGEQLRILEQ SGEWWKAQSL TTGQEGFIPF
121 NFVAKANSLE PEPWFFKNLS RKDAERQLLA PGNTHGSFLI RESESTAGSF SLSVRDFDQN
181 QGEVVKHYKI RNLDNGGFYI SPRITFPGLH ELVRHYTNAS DGLCTRLSRP CQTQKPQKPW
241 WEDEWEVPRE TLKLVERLGA GQFGEVVMGY YNGHTKVAVK SLKQGSMSPD AFLAEANLMK
301 QLQHQRLLVRL YAVVTQEPIY IITEYMENGS LVDFLKTSPG IKLTINKLLD MAAQIAEGMA
361 FIEERNYIHR DLRAANILVS DTLSCKIADF GLARLIEDNE YTAREGAKFP IKWTAPEAIN
421 YGFTTIKSDV WSGILLTEI VTHGRIPYPG MTNPEVIQNL ERGYRMVRPD NCPEELYQLM
481 RLCWKERPED RPTFDYLRSV LEDFFTATEG QYQPQP
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Recombinant Lck nucleotide sequence:

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1 atgcatcacc atcaccatca tatgggctgt ggctgcagct cacaccgga agatgactgg
61 atggaaaaca tcgatgtgtg tgagaactgc cattatccca tagtcccact ggatggcaag
121 ggcacgctgc tcatccgaaa tggctctgag gtgcgggacc cactggttac ctacgaaggc
181 tccaatccgc cggcttcccc actgcaagac aacctggta tcgctctgca cagctatgag
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481 cgggagagcg agagcaccgc gggatcgttt tcaactgctcg tccgggactt cgaccagaac
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