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Certificate of Analysis

Lck, active

(Recombinant enzyme expressed in Sf21 insect cells)

Catalogue # 14-442

Lot # 1626267 from Bulk # 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# 1626267): 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.

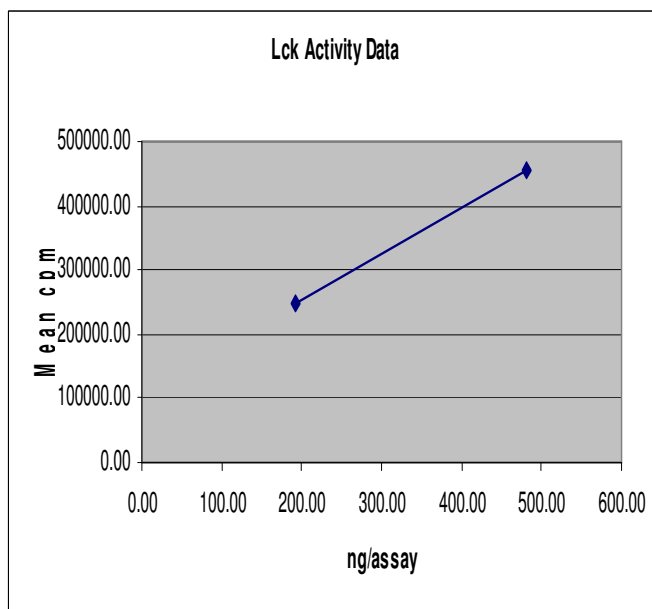
Storage and Stability: Stable for 1 year at -70°C from date of shipment. For maximum recovery of product, centrifuge original vial prior to removing the cap.

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.

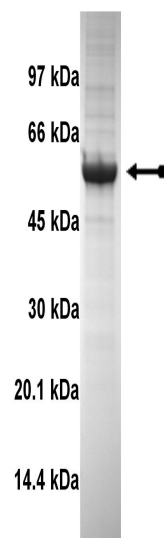
**FOR IN VITRO RESEARCH USE ONLY
NOT FOR USE IN HUMANS OR ANIMALS**

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 three.



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

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 per assay point.
3. **Lck, active:** 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 **cdc2**.
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 with cpm of control samples that contain all assay components plus 1µl of 30% phosphoric acid (background cpm.)

Lck Sequence Information

Protein Human Lck
Tags N-terminal 6His
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 VRDPLVTYEG
61 SNPPASPLQD NLVIALHSYE PSHDGDLDGFE KGEQLRILEQ SGEWWKAQSL TTGQEGFIPF
121 NFVAKANSLE PEPWFFKNLS RKDAERQLLA PGNTHGSFLI RESESTAGSF SLSVRDFDQN
181 QGEVVKHYKI RNLDNGGFYI SPRITFPGLH ELVRHYTNAS DGLCTRLSRP CQTQKPQKPW
241 WEDEWEVPRE TLKLVERLGA GQFGEVWMGY YNGHTKVAVK SLKQGSMSPD AFLAEANLMK
301 QLQHQRLLVRL YAVVTQEPIY IITEYMENG LVDFLKTPSG IKLTINKLLD MAAQIAEGMA
361 FIEERNYIHR DLRAANILVS DTLSCKIADF GLARLIEDNE YTAREGAKFP IKWTAPEAIN
421 YGTFTIKSDV WSFGILLTEI VTHGRIPYPG MTNPEVIQNL ERGYRMVRPD NCPEELYQLM
481 RLCWKERPED RPTFDYLRVS 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 cattatcca tagtcccact ggatggcaag
121 ggcacgctgc tcatccgaaa tggctctgag gtgcgggacc cactggttac ctacgaaggc
181 tccaatccgc cggcttcccc actgcaagac aacctggtta tcgctctgca cagctatgag
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481 cgggagagcg agagcaccgc gggatcgttt tcaactgtcg tccgggactt cgaccagaac
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1441 aggctgtgct ggaaggagcg cccagaggac cggcccacct ttgactacct gcgcatgtg
1501 ctggaggact tcttcacggc cacagagggc cagtaccagc ctcagccttg a
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Reviewed and approved by site quality representative.

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