

Certificate of Analysis

Lck, activated

(Recombinant enzyme expressed in Sf21 insect cells)

Item # 14-842, 14-842-K, 14-842M

Parent Lot # D8KN038N

The data presented in this document apply to the parent lot shown above and to all pack sizes derived from subsequent vialling runs of this parent lot. An alphabetical suffix after the parent lot number is used to denote each vialling run.

Product Description: N-terminal 6His-tagged, recombinant, human Lck full length, expressed by baculovirus in Sf21 insect cells. Purified using Ni²⁺/NTA agarose. Auto-activated on column by incubating with Mg/ATP, excess ATP and MgAc removed by multiple column wash steps. Purity 99% by SDS-PAGE and Coomassie blue staining. MW = 59kDa.

Specific Activity (Parent lot# D8KN038N): 3057U/mg, where one unit of Lck, activated activity is defined as 1nmol phosphate incorporated into 250µM (KVEKIGEGTYGVVYK) per minute at 30°C with a final ATP concentration of 100µM.

Formulation: 2.184mg/ml of enzyme in 50mM Tris/HCl pH7.5, 300mM NaCl, 0.1mM EGTA, 0.03% Brij-35, 5% glycerol, 1mM benzamidine, 0.2mM PMSF, 0.1% 2-mercaptoethanol, 5mM β-glycerophosphate, 1mM Na₃VO₄. Frozen solution.

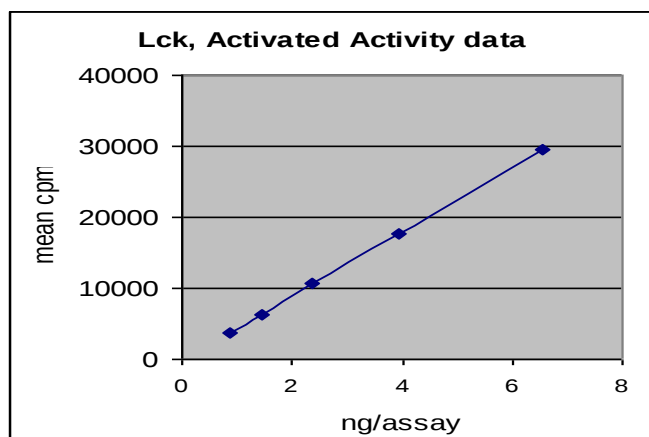
Storage and Stability: On receipt of material store at -70°C. Unopened reagent is stable for a minimum of 1 year from date of shipment when stored at recommended storage temperature. Avoid repeat freeze/thaw cycles. For maximum recovery of product, centrifuge original vial prior to removing the cap.

Handling Recommendations: Rapidly thaw the vial under cold water and immediately place on ice. Aliquot unused material into pre-chilled micro-centrifuge 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**

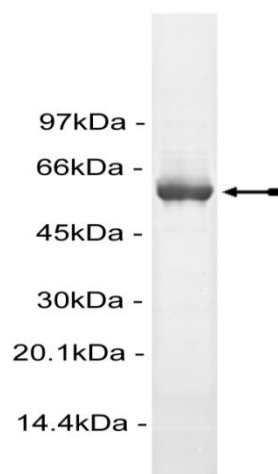
Quality Control Testing

Kinase Assay: 0.86–6.57ng of this lot of enzyme phosphorylated 250µM (KVEKIGEGTYGVVYK) 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 product identity as Lck with the translated sequence listed on page three.

SDS-PAGE and Coomassie Stain: Purity was assessed by SDS-PAGE and Coomassie blue staining using 3µg of Lck, activated.



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Kinase Assay Protocol

Stock Solutions:

1. **5 x Reaction Buffer:** 40mM MOPS/NaOH pH7.0, 1mM EDTA.
2. **(KVEKIGEGTYGVVYK):** Use at a final assay concentration of 250µM. Prepare a 2.5mM stock and add 2.5µl of stock per assay point.
3. **Lck, activated:** Dilute with 20mM MOPS/NaOH pH7.0, 1mM EDTA, 0.01% Brij-35, 5% glycerol, 0.1% 2-mercaptoethanol, 1mg/ml BSA. Use 0.86–6.57ng 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 5µl of 5 x reaction buffer per assay to wells.
2. Add 2.5µl of **(KVEKIGEGTYGVVYK)**.
3. Add **2.5µl (0.86–6.57ng) Lck, activated**.
4. Add 5µl 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 three times 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.

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Lck Sequence Information

Protein	Human Lck
Tags	N-terminal 6His
Native sequence	M8 of the recombinant protein is equivalent to M1 of human Lck
Accession number	GenBank NM_005356 The translated product of NM_005356 is identical to the amino acid sequence described in Swiss prot P06239.

Recombinant Lck amino acid sequence:

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1  MHHHHHHMGC  GCSSHPEDDW  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  GQFGEVWMGY  YNGHTKVAVK  SLKQGSMSPD  AFLAEANLMK
301  QLQHQRRLVRL  YAVVTQEPIY  IITEYMENG  S  LVDFLKTSPG  IKLTINKLLD  MAAQIAEGMA
361  FIEERNYIHR  DLRAANILVS  DTLCKIADF  GLARLIEDNE  YTAREGAKFP  IKWTAPEAIN
421  YGTFTIKSDV  WSGGILLTEI  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  cattatccca  tagtcccact  ggatggcaag
121  ggcacgctgc  tcatccgaaa  tggctctgag  gtgcgggacc  cactggttac  ctacgaaggc
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