eurofins | Discovery Services

Certificate of Analysis

Lck, active

(Recombinant enzyme expressed in Sf21 insect cells)
Item # 14-442, 14-442-K, 14-442M
Parent Lot # WAE0125

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 full-length human Lck expressed in Sf21 insect cells. Purified using Ni^{2+}/NTA agarose. Purity 96% by SDS-PAGE and Coomassie blue staining. MW = 59kDa.

Specific Activity (Parent lot# WAE0125): 394U/mg, where one unit of Lck, active 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: 0.99mg/ml of enzyme in 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.

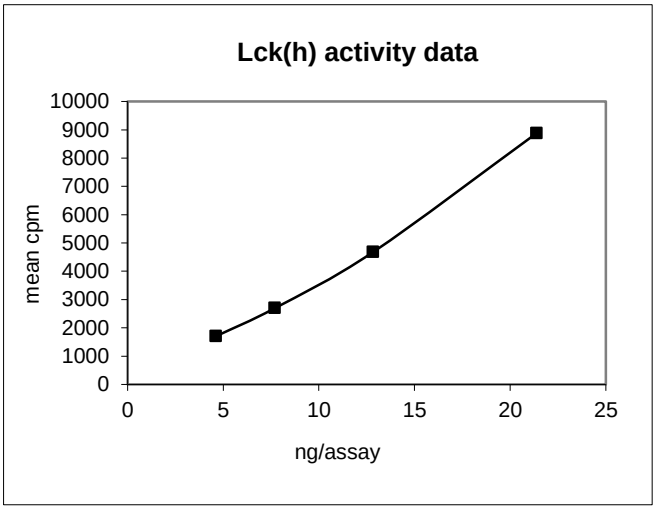
Storage and Stability: On receipt of material store at -70°C. Unopened reagent is stable for a minimum of 1 year from date of shipment when stored at recommended storage temperature. Avoid repeat freeze/thaw cycles. For maximum recovery of product, centrifuge original vial prior to removing the cap.

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.

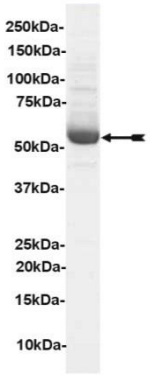
FOR IN VITRO RESEARCH USE ONLY
NOT FOR USE IN HUMANS OR ANIMALS

Quality Control Testing

Kinase Assay: 4.62–21.38ng 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 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, active.

Eurofins Pharma Discovery Services UK Limited
Gemini Crescent
Dundee Technology Park
DUNDEE
DD2 1SW United Kingdom
T | +44 (0)1382 561600
F | +44 (0)1382 561601
www.eurofins.com/pharmadiscovery

Certificate of Analysis

Kinase Assay Protocol

Stock Solutions:

1. **4 x Reaction Buffer:** 200mM Tris/HCl pH7.5, 0.4mM EGTA, 0.4mM Na_3VO_4.
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 4.62–21.38ng per assay point.
4. **[γ-^{33}P]ATP:** 2.5 x magnesium acetate/[γ-^{33}P]ATP cocktail: 25mM MgAc and 0.25mM ATP to which is added [γ-^{33}P]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 (4.62–21.38ng) Lck, active**.
4. Add 3.75µl of dH_2O.
5. Add 10µl of diluted [γ-^{33}P]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.)

eurofins | Discovery Services

Certificate of Analysis

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:

```
   1 MHHHHHHMGC GCSSHPEDDW MENIDVCENC HYPIVPLDGK GTLLIRNGSE VRDPLVTYEG
  61 SNPPASPLQD NLVIALHSYE PSHDGDLGFE 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 QLQHQRLVRL YAVVTQEPIY IITEYMENGS LVDFLKTPSG IKLTINKLLD MAAQIAEGMA
 361 FIEERNYIHR DLRAANILVS DTLSCKIADF GLARLIEDNE YTAREGAKFP IKWTAPEAIN
 421 YGTFTIKSDV WSFGILLTEI VTHGRIPYPG MTNPEVIQNL ERGYRMVRPD NCPEELYQLM
 481 RLCWKERPED RPTFDYLRSV LEDFFTATEG QYQPQP
```

Recombinant Lck nucleotide sequence:

```
   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 aacctggtta tcgctctgca cagctatgag
 241 ccctctcacg acggagatct gggctttgag aaggggggaac agctccgcat cctggagcag
 301 agcggcgagt ggtggaaggc gcagtccctg accacgggcc aggaaggctt catccccttc
 361 aattttgtgg ccaaagcgaa cagtctggag cccgaaccct ggttcttcaa gaacctgagc
 421 cgcaaggacg cggagcggca gctcctggcg cccgggaaca ctcacggctc cttcctcatc
 481 cgggagagcg agagcaccgc gggatcgttt tcactgtcgg tccgggactt cgaccagaac
 541 cagggagagg tggtgaaaca ttacaagatc cgtaatctgg acaacggtgg cttctacatc
 601 tcccctcgaa tcacttttcc cggcctgcat gaactggtcc gccattacac caatgcttca
 661 gatgggctgt gcacacggtt gagccgcccc tgccagaccc agaagcccca gaagccgtgg
 721 tgggaggacg agtgggaggt tcccagggag acgctgaagc tggtggagcg gctgggggct
 781 ggacagttcg gggaggtgtg gatggggtac tacaacgggc acacgaaggt ggcggtgaag
 841 agcctgaagc agggcagcat gtccccggac gccttcctgg ccgaggccaa cctcatgaag
 901 cagctgcaac accagcggct ggttcggctc tacgctgtgg tcacccagga gcccatctac
 961 atcatcactg aatacatgga gaatgggagt ctagtggatt ttctcaagac cccttcaggc
1021 atcaagttga ccatcaacaa actcctggac atggcagccc aaattgcaga aggcatggca
1081 ttcattgaag agcggaatta tattcatcgt gaccttcggg ctgccaacat tctggtgtct
1141 gacaccctga gctgcaagat tgcagacttt ggcctagcac gcctcattga ggacaacgag
1201 tacacagcca gggaggggggc caagtttccc attaagtgga cagcgccaga agccattaac
1261 tacgggacat tcaccatcaa gtcagatgtg tggtcttttg ggatcctgct gacggaaatt
1321 gtcacccacg gccgcatccc ttacccaggg atgaccaacc cggaggtgat tcagaacctg
1381 gagcgaggct accgcatggt gcgccctgac aactgtccag aggagctgta ccaactcatg
1441 aggctgtgct ggaaggagcg cccagaggac cggcccacct ttgactacct gcgcagtgtg
1501 ctggaggact tcttcacggc cacagagggc cagtaccagc ctcagccttg a
```

Reviewed and approved by site quality representative.