

Standard Operating Procedure

Preparation of active Lck [2 - 539]

<u>Enzyme description:-</u>	Lck [2 - 539]
<u>Clone number:-</u>	DU 567
<u>Source:-</u>	Recombinant
<u>Expression system:-</u>	Baculovirus expression vector system (BEVS)/Insect cells
<u>Tag:-</u>	N-terminal His(6)
<u>Purification method:-</u>	Ni ²⁺ -NTA agarose.
<u>Expression level:-</u>	2 mg/L
<u>Molecular mass:-</u>	64, 734 daltons
<u>Purity:-</u>	>85%
<u>Activation protocol:-</u>	Constitutively active

Enzyme storage buffer:-

50 mM Tris-HCl pH 7.5, 50 % glycerol, 150 mM NaCl, 0.1 mM EGTA, 0.1 % 2-mercaptoethanol, 0.02 % Brij-35, 0.2 mM PMSF, 1 mM Benzamidine.

Storage temperature:- -20 °C

Assay:- Standard filter binding assay

Assay buffer:-

50 mM Tris-HCl pH 7.5, 0.1 % 2-mercaptoethanol, 0.1 mM EGTA, 10 mM magnesium acetate

Substrate:-

KVEKIGEGTYGVVYK [Residues 5 – 20 of human CDK2]
Final concentration: 250 μ M

Specific activity range:- 700 – 1400 U/mg

University of Dundee

Clone Data Sheet - Lck [2 - 539]

Protein Lck [2 - 539]

Clone number DU 567

Species Mouse

Accession no I48845

Tags N-terminal His(6)

Baculovirus expressed protein MSYYHHHHHDYDIPTTENLYFQGAMDPEFGCGCSSHPEDDWME
NIDVCENCHYPIVPLDSKISLPIRNGSEVRDPLVTYEGSLPPAS
PLQDNLVIALHSYEPSHDGDLGFEEKGEQLRILEQSGEWWKAQSL
TTGQEGFIPFNFVAKANSLEPEPWFFKNLSRKDAERQLLAPGNT
HGSFLIRESESTAGSFSLSVRDFDQNGEVVKHYKIRNLDNGGF
YISPRITFPGLHDLVRHYTNASDGLCTKLSRPCQTQKPQKPWE
DEWEVPRETLKLVERLGAGQFGEVWMGYNGHTKVAVKSLKQGS
MSPDAFLAEANLMKQLQHPRLVRLYAVVTQEPYIITEYMENG
LVDFLKTSPSGIKLVNKLDDMAAQIAEGMAFIEEQNYIHRDLRA
ANILVSDTLCKIADFGLARLIEDNEYTAREGAKFPIKWTAP
INYGTFTIKSDVWSFGILLTEIVTHGRIPYPGMTNPEVIQNL
GYRMVRPDNCPEELYHLMMLCWKERPEDRPTFDYLRSLDDFFT
ATEGQYQPPVDL

Native sequence Amino acids G2 – L539 of mouse Lck.
Residue G31 of the fusion protein is equivalent to G2 of mouse Lck. The His(6) tag is located at residues 5 – 10.

Protease cleavage rTEV (ENLYFQG) residues 18 - 24

Cloning sites *Eco*R1 and *Not*I sites of pFastBAC HTa

**Nucleotide
sequence of insert**

GAATTCGGCTGTGGCTGCAGCTCACACCCTGAAGATGACTGGATGGAGAA
CATTGACGTGTGTGAAAAC TGCCACTATCCCATAGTCCC ACTGGACAGCA
AGATCTCGCTGCCCATCCGGAATGGCTCTGAAGTGC GGGGACCCACTGGTTC
ACCTATGAGGGATCTCTCCCACCAGCATCCCCGCTGCAAGACAACCTGGT
TATCGCCCTGCACAGTTATGAGCCCTCCCATGATGGAGACTTGGGCTTTG
AGAAGGGTGAACAGCTCCGAATCCTGGAGCAGAGCGGTGAGTGGTGGAAG
GCTCAGTCCCTGACGACTGGCCAAGAAGGCTTCATTCCCTTCAACTTCGT
GGCGAAAGCAAACAGCCTGGAGCCTGAACCTTG GTTCTTCAAGAATCTGA
GCCGTAAGGACGCCGAGCGGCAGCTTTTGGCGCCCGGGAACACGCATGGA
TCCTTCCTGATCCGGGAAAGCGAAAGCACTGCGGGGTCTTTTCCCTGTC
GGTCAGAGACTTCGACCAGAACCAGGGAGAAGTGGTGAAACATTACAAGA
TCCGTAACCTAGACAACGGTGGCTTCTACATCTCCCTCGTATCACTTTT
CCCGGATTGCACGATCTAGTCCGCCATTACACCAACGCCTCTGATGGGCT
GTGCACAAAGTTGAGCCGTCCTTGCCAGACCCAGAAGCCCCAGAAACCAT
GGTGGGAGGACGAATGGGAAGTTCCCAGGGAAACACTGAAGTTGGTGAG
CGGCTGGGAGCTGGCCAGTTCGGGGAAAGTGTGGATGGGGTACTACAACGG
ACACACGAAGGTGGCGGTGAAGAGTCTGAAACAAGGGAGCATGTCCCCCG
ACGCCTTCCTGGCTGAGGCTAACCTCATGAAGCAGCTGCAGCACCCGCGG
CTAGTCCGGCTTTATGCAGTGGTCACCCAGGAACCCATCTACATCATCAC
GGAATACATGGAGAACGGGAGCCTAGTAGATTTTCTCAAGACTCCCTCGG
GCATCAAGTTGAATGTCAACAAACTTTTGGACATGGCAGCCCAGATTGCA
GAGGGCATGGCGTTCATCGAAGAACAGAATTACATCCATCGGGACCTGCG
CGCCGCCAACATCCTGGTGTCTGACACGCTGAGCTGCAAGATTGCAGACT
TTGGCCTGGCGCGCCTCATTGAGGACAATGAGTACACGGCCCCGGGAGGGG
GCCAAATTTCCCATTAAGTGGACAGCACCAGAAGCCATTA ACTATGGGAC
CTTCACCATCAAGTCAGACGTGTGGTCCTTCGGGATCTTGCTTACAGAGA
TCGTCACCCACGGTCAATCCCTTACCCAGGAATGACCAACCCTGAAGTC
ATTCAGAACCTGGAGAGAGGCTACCGCATGGTGAGACCTGACA ACTGTCC
GGAAGAGCTGTACCACCTCATGATGCTGTGCTGGAAGGAGCGCC CAGAGG
ACCGGCCACGTTTGA CTACCTTCGGAGTGTTCTGGATGACTTCTT CACA
GCCACAGAGGGCCAGTACCAGCCCCAGCCTGTCGACCTCtaggcggccgc