*Division of Signal Tranduction Therapy*

# Standard Operating Procedure

# Preparation of active Lck [2 - 509]

**Enzyme description:-** Lck [2 - 509]

**Clone number:-** DU 567

**Source:-** Recombinant

**Expression system:-** Baculovirus expression vector system

**Tag:-** N-terminal His(6)

**Purification method:-** $Ni^{2+}$-NTA agarose

**Expression level:-** 2 mg/L

**Calculated molecular mass:-** 64, 734 daltons

**Purity:-** >85 %

**Activation protocol:-** 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 MgAc

**Substrate:-**
KVEKIGEGTYGVVYK [Residues 5 – 20 of human CDK2]
Final concentration: 250 µM

**Specific activity range:-** 700 – 1400 U/mg

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# Clone Data Sheet

## Lck [2 - 509]

**Protein** Lck [2 - 509]

**Clone number** DU 567

**Species** Mouse

**Accession no** X03533

**Tags** N-terminal His(6)

**Baculovirus expressed protein**

MSYYHHHHHHDYDIPTTENLYFQGAMDPEF**GCGCSSHPEDDWME**
**NIDVCENCHYPIVPLDSKISLPIRNGSEVRDPLVTYEGSLPPAS**
**PLQDNLVIALHSYEPSHDGDLGFEKGEQLRILEQSGEWWKAQSL**
**TTGQEGFIPFNFVAKANSLEPEPWFFKNLSRKDAERQLLAPGNT**
**HGSFLIRESESTAGSFSLSVRDFDQNQGEVVKHYKIRNLDNGGF**
**YISPRITFPGLHDLVRHYTNASDGLCTKLSRPCQTQKPQKPWWE**
**DEWEVPRETLKLVERLGAGQFGEVWMGYYNGHTKVAVKSLKQGS**
**MSPDAFLAEANLMKQLQHPRLVRLYAVVTQEPIYIITEYMENGS**
**LVDFLKTPSGIKLNVNKLLDMAAQIAEGMAFIEEQNYIHRDLRA**
**ANILVSDTLSCKIADFGLARLIEDNEYTAREGAKFPIKWTAPEA**
**INYGTFTIKSDVWSFGILLTEIVTHGRIPYPGMTNPEVIQNLER**
**GYRMVRPDNCPEELYHLMMLCWKERPEDRPTFDYLRSVLDDFFT**
**ATEGQYQPQP**VDL

**Native sequence**

Amino acids G2 – P509 (end) 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. The following sequence is present after the Lck sequence, VDL, residues 539 – 541.

The following amino acid substitutions are present:
V – G, where V4 of the native sequence is G33 of the fusion protein.
N – H, where N8 of the native sequence is H37 of the fusion protein.

**Protease cleavage** rTEV (ENLYFQG) residues 18 - 24

**Cloning sites** *Eco*R1 and *Not*1 sites of pFastBAC HTa

**Nucleotide sequence of insert**

GAATTCGGCTGTGGCTGCAGCTCACACCCTGAAGATGACTGGATGGAGAA
CATTGACGTGTGTGAAAACTGCCACTATCCCATAGTCCCACTGGACAGCA
AGATCTCGCTGCCCATCCGGAATGGCTCTGAAGTGCGGGACCCACTGGTC
ACCTATGAGGGATCTCTCCCACCAGCATCCCCGCTGCAAGACAACCTGGT
TATCGCCCTGCACAGTTATGAGCCCTCCCATGATGGAGACTTGGGCTTTG
AGAAGGGTGAACAGCTCCGAATCCTGGAGCAGAGCGGTGAGTGGTGGAAG
GCTCAGTCCCTGACGACTGGCCAAGAAGGCTTCATTCCCTTCAACTTCGT
GGCGAAAGCAAACAGCCTGGAGCCTGAACCTTGGTTCTTCAAGAATCTGA
GCCGTAAGGACGCCGAGCGGCAGCTTTTGGCGCCCGGGAACACGCATGGA
TCCTTCCTGATCCGGGAAAGCGAAAGCACTGCGGGGTCCTTTTCCCTGTC
GGTCAGAGACTTCGACCAGAACCAGGGAGAAGTGGTGAAACATTACAAGA
TCCGTAACCTAGACAACGGTGGCTTCTACATCTCCCCTCGTATCACTTTT
CCCGGATTGCACGATCTAGTCCGCCATTACACCAACGCCTCTGATGGGCT
GTGCACAAAGTTGAGCCGTCCTTGCCAGACCCAGAAGCCCCAGAAACCAT
GGTGGGAGGACGAATGGGAAGTTCCCAGGGAAACACTGAAGTTGGTGGAG
CGGCTGGGAGCTGGCCAGTTCGGGGAAGTGTGGATGGGGTACTACAACGG
ACACACGAAGGTGGCGGTGAAGAGTCTGAAACAAGGGAGCATGTCCCCCG
ACGCCTTCCTGGCTGAGGCTAACCTCATGAAGCAGCTGCAGCACCCGCGG
CTAGTCCGGCTTTATGCAGTGGTCACCCAGGAACCCATCTACATCATCAC
GGAATACATGGAGAACGGGAGCCTAGTAGATTTTCTCAAGACTCCCTCGG
GCATCAAGTTGAATGTCAACAAACTTTTGGACATGGCAGCCCAGATTGCA
GAGGGCATGGCGTTCATCGAAGAACAGAATTACATCCATCGGGACCTGCG
CGCCGCCAACATCCTGGTGTCTGACACGCTGAGCTGCAAGATTGCAGACT
TTGGCCTGGCGCGCCTCATTGAGGACAATGAGTACACGGCCCGGGAGGGG
GCCAAATTTCCCATTAAGTGGACAGCACCAGAAGCCATTAACTATGGGAC
CTTCACCATCAAGTCAGACGTGTGGTCCTTCGGGATCTTGCTTACAGAGA
TCGTCACCCACGGTCGAATCCCTTACCCAGGAATGACCAACCCTGAAGTC
ATTCAGAACCTGGAGAGAGGCTACCGCATGGTGAGACCTGACAACTGTCC
GGAAGAGCTGTACCACCTCATGATGCTGTGCTGGAAGGAGCGCCCAGAGG
ACCGGCCCACGTTTGACTACCTTCGGAGTGTTCTGGATGACTTCTTCACA
GCCACAGAGGGCCAGTACCAGCCCCAGCCTGTCGACCTCtaggcggccgc