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Recombinant full length human Lck kinase containing N-terminal GST tag

Catalog No.	CRL102A CRL102B	**Quantity:**	5 µg 20 µg
Alternate Names:	p56lck		

Description: Recombinant full length human Lck containing N-terminal GST tag was expressed by baculovirus in Sf 9 insect cells.

Lck (p56lck) is a member of the src family of non-receptor tyrosine kinases. It was identified as a gene rearranged and overexpressed in the murine lymphoma LSTRA, most likely as a result of the insertion of Moloney mouse leukemia virus DNA immediately adjacent to the gene. Lck behaves as a proto-oncogene and can lead to cell transformation upon activation. A number of human cancer cell lines show overexpression of Lck, pointing to a possible role for this kinase in the initiation and maintenance of the transformed state in human cancers. Colon cancers and T-cell leukemias frequently show defective regulation of Lck expression and activity. Inappropriate T cell activation and proliferation have been identified as an early event in auto-immune disease. Lck plays a prominent role in T-cell development, activation, proliferation and survival. Lck is coupled to both the CD4 and CD8 antigens (which serve as receptors for nonpolymorphic regions of products of the major histocompatibility complex and have been implicated in the regulation of T-cell growth) in T-cells and phosphorylates CD3. Lck phosphorylates many cellular protein substrates as a result of T-cell receptor signaling cascade. This includes phosphorylation of proteins such as Ras GTPase-activating protein (RasGAP) and two RasGAP-associated proteins, p56(dok) and p62(dok).

Concentration: 0.1 mg/ml

Gene Accession No: NM_005356

Source: Sf9 insect cells

Formulation: Recombinant protein in storage buffer (50 mM Tris-HCl + 150 mM NaCl + 0.25 mM DTT + 0.1 mM EGTA + 0.1 mM EDTA + 0.1 mM PMSF + 30% glycerol; pH 7.5).

Purity: 1 µg of Lck protein was subjected to SDS-PAGE and Coomassie blue staining. The scan of the gel showed >90% purity of the Lck product, and the band was at ~84 kDa (Fig. 2).

Specific Activity: 239 nmol/min/mg: 239 nmol phosphate incorporated into MBP per minute per mg protein at 30ºC for 15 minutes using a final concentration of 50 µM ATP (0.83 µCi/assay). See QA/QC section for details.

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Storage & Stability: Store product frozen at or below -70ºC. Stable for 1 year at -70ºC as undiluted stock. Aliquot to avoid repeated thawing and freezing.

QA/QC: Lck protein (100 ng/µl concentration) was diluted to 20 ng/µl with assay dilution buffer (4 mM MOPS + 2.5 mM β-glycerophosphate + 1 mM EGTA + 0.4 mM EDTA + 4 mM $MgCl_2$ + 0.05 mM DTT; pH 7.2), followed by 2-fold serial dilutions, and then the 10µl diluted proteins were used to phosphorylate the myelin basic protein (MBP) in the following assay condition:

- 10 µl diluted Lck protein
- 5 µl MBP (1 mg/ml stock)
- 5 µl water
- 5 µl [^{32}P] ATP mixture (250 µM ATP, 0.16 µCi/µl in 4x assay dilution buffer)

The various reaction components, except [^{32}P] ATP, were incubated at 30ºC and the reaction started by the addition of [^{32}P] ATP. After 15 minutes, the reaction was terminated by spotting 20 µl of the reaction mixture onto a phosphocellulose P81 paper. The P81 paper was dried and washed several times in 1% phosphoric acid prior to counting in the presence of scintillation fluid in a scintillation counter. The actual counts, using various dilutions of the enzyme in the assay, are shown in Fig. 1.

Fig. 1 LCK activity assay

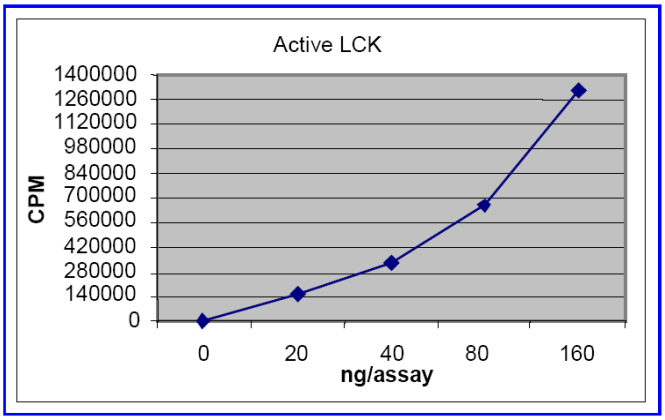
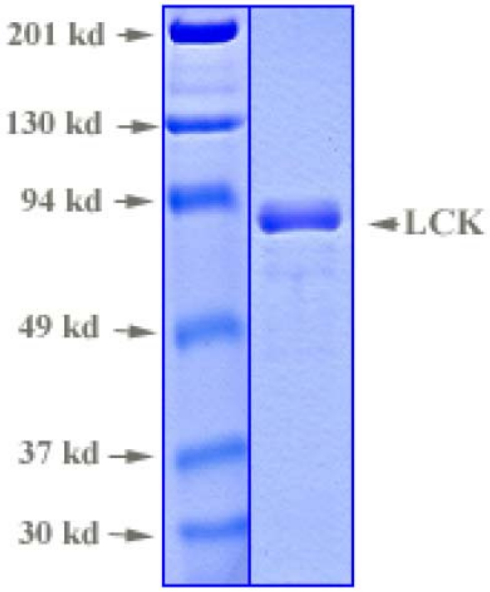


Fig. 2 LCK protein gel



Please note: always centrifuge vials before opening.

NOT FOR HUMAN USE. FOR RESEARCH ONLY. NOT FOR DIAGNOSTIC OR THERAPEUTIC USE.

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