

# Recombinant Human Active Lck

## Certificate of Analysis

Catalog Number: 3704-KS

Lot Number: 1466480

### Specifications and Use

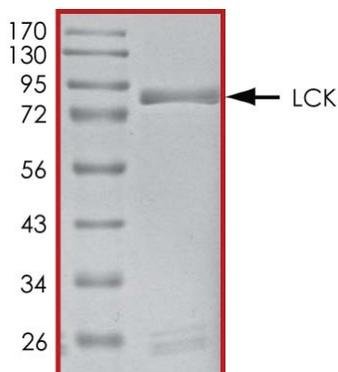
- Source** ♦ Recombinant full-length human Lck was expressed by baculovirus in *Sf9* insect cells using an N-terminal GST tag. The gene accession number is NM\_005356.
- Molecular Mass** ♦ The approximate molecular weight is 84 kDa (see Figure 1 below).
- Purity** ♦ The purity was determined to be > 95% by densitometry (see Figure 1 below).
- Formulation** ♦ Supplied in 50 mM Tris-HCl, pH 7.5, 150 mM NaCl, 10 mM glutathione, 0.25 mM DTT, 0.1 mM EDTA, 0.1 mM PMSF, 25% glycerol.
- Size** ♦ 10 µg.
- Concentration** ♦ 0.1 µg/µL.
- Activity** ♦ The specific activity of Lck was determined to be 180 nmol/min/mg using a Poly (Glu:Tyr, 4:1) synthetic peptide substrate (see Activity Assay Protocol).
- Storage** ♦ This product is stable at ≤ -70 °C for up to 1 year from the date of receipt. For optimal storage, aliquot into smaller quantities after centrifugation and store at recommended temperature.  
♦ **Avoid repeated freeze-thaw cycles.**

### Lck

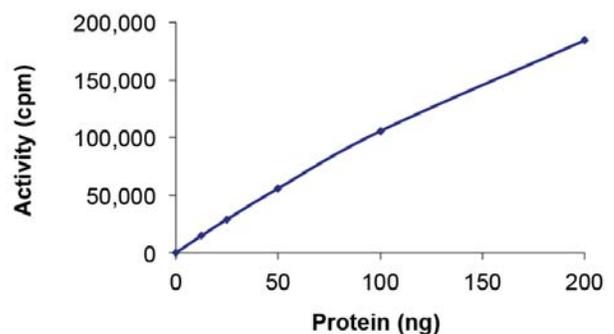
Lck (p56lck) is a member of the Src family of non-receptor tyrosine kinases. It was identified as a gene rearranged and over-expressed in the murine lymphoma LSTRA, most likely as a result of the insertion of Moloney murine leukemia virus DNA immediately adjacent to the gene (1). Lck behaves as a proto-oncogene and can lead to cell transformation upon activation. A number of human cancer cell lines show over-expression of Lck, pointing to a possible role for this kinase in the initiation and maintenance of the transformed state in human cancers (2).

### References

1. Fischer, S. *et al.* (1987) *Biochem. Biophys. Res. Commun.* **143**:819.
2. Veillette, A. *et al.* (1987) *Oncogene Res.* **1**:357.



**Figure 1:** SDS-PAGE and Coomassie stain. The approximate molecular weight is 84 kDa and the purity is > 95%.



**Figure 2:** Enzymatic assay results. The specific activity of Lck was determined to be 180 nmol/min/mg as per activity assay

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protocol (on reverse).

## Activity Assay Protocol

### Solutions Required

- **Active Kinase** - Active Lck (0.1 µg/µL) diluted with Kinase Dilution Buffer and assayed as outlined in Figure 2.  
**Note:** These are suggested working dilutions. Optimal dilutions should be determined by each laboratory for each application.
- **Kinase Assay Buffer I, pH 7.2** - 25 mM MOPS, 12.5 mM β-glycerolphosphate, 25 mM MgCl<sub>2</sub>, 5 mM EGTA, 2 mM EDTA. Add 0.25 mM DTT to the Kinase Assay Buffer prior to use.
- **Kinase Dilution Buffer, pH 7.2** - Kinase Assay Buffer I diluted at a 1:4 ratio (5-fold dilution) with 50 ng/µL BSA solution.
- **10 mM ATP Stock Solution** - Prepare the ATP Stock Solution by dissolving 55 mg of ATP in 10 mL of Kinase Assay Buffer I.
- **[<sup>33</sup>P]-ATP Assay Cocktail** - Prepare 250 µM [<sup>33</sup>P]-ATP Assay Cocktail in a designated radioactive work area by combining 150 µL of 10 mM ATP Stock Solution, 100 µL of [<sup>33</sup>P]-ATP (1 mCi/100 µL), and 5.75 mL of Kinase Assay Buffer I.
- **Substrate** - Poly (Glu:Tyr, 4:1) synthetic peptide substrate diluted in distilled or deionized water to a final concentration of 1.0 mg/mL.

### Assay Procedure

1. Thaw the [<sup>33</sup>P]-ATP Assay Cocktail in a shielded container in a designated radioactive work area.
2. Thaw the Active Lck, Kinase Assay Buffer I, Substrate, and Kinase Dilution Buffer on ice.
3. In a pre-cooled microfuge tube, add the following reaction components bringing the initial reaction volume up to 20 µL.

Reaction Component	Amount
Diluted Active Lck	10 µL
Substrate (1 mg/mL Stock Solution)	5 µL
Distilled Water (4 °C)	5 µL

4. Set up the blank control as outlined in step 3, excluding the addition of the substrate. Replace the substrate with an equal volume of distilled or deionized water.
5. Initiate the reaction by the addition of 5 µL [<sup>33</sup>P]-ATP Assay Cocktail, bringing the final volume up to 25 µL. Incubate the mixture in a water bath at 30 °C for 15 minutes.
6. After the 15 minute incubation period, terminate the reaction by spotting 20 µL of the reaction mixture onto individual pre-cut strips of phosphocellulose P81 paper.
7. Air dry the pre-cut P81 strip and sequentially wash in a 1% phosphoric acid solution (dilute 10 mL of phosphoric acid and make a 1 liter solution with distilled water) with constant gentle stirring. It is recommended that the strips be washed a total of three times for approximately 10 minutes each.
8. Count the radioactivity on the P81 paper in the presence of scintillation fluid in a scintillation counter.
9. Determine the corrected cpm by removing the blank control value (see step 4) for each sample and calculate the kinase specific activity as outlined below.

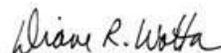
### Calculation of [<sup>33</sup>P]-ATP Specific Activity (SA) (cpm/pmol)

Specific Activity (SA) = cpm for 5 µL [<sup>33</sup>P]-ATP/pmole of ATP (in 5 µL of a 250 µM ATP stock solution, *i.e.* 1250 pmoles)

### Calculation of Kinase Specific Activity (SA) (pmol/minutes/µg or nmol/minutes/mg)

Corrected cpm from reaction / [(SA of <sup>33</sup>P-ATP in cpm/pmol) x (Reaction time in minutes) x (Enzyme amount in µg or mg)] x [(Reaction volume) / (Spot Volume)]

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