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1-866-9 KINASE (54-6273)

Catalog #	Aliquot Size
L03-10G-05	5 µg
L03-10G-10	10 µg

LCK, Active

Full-length human recombinant protein expressed in Sf9 cells

Catalog # L03-10G
Lot # C2007-6

Product Description

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.

Gene Aliases

YT16, p56lck, pp58lck

Formulation

Recombinant protein stored in 50mM Tris-HCl, pH 7.5, 150mM NaCl, 10mM glutathione, 0.1mM EDTA, 0.25mM DTT, 0.1mM PMSF, and 25% glycerol.

Storage and Stability

Store product at –70°C. For optimal storage, aliquot target into smaller quantities after centrifugation and store at recommended temperature. For most favorable performance, avoid repeated handling and multiple freeze/thaw cycles.

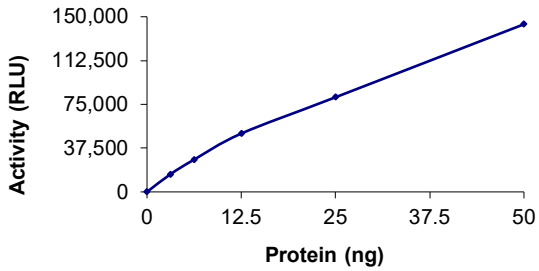
Scientific Background

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 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 overexpression of LCK, pointing to a possible role for this kinase in the initiation and maintenance of the transformed state in human cancers (2).

References

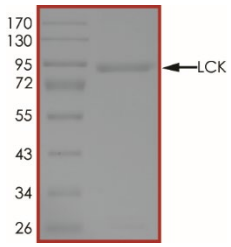
1. Fischer, S. et al: The amino terminal region of the p56 lck from LSTRA exerts negative modulation on the tyrosine kinase activity. Biochem Biophys Res Commun. 1987 Mar 30; 143(3):819-26.
2. Veillette, A. et al: Expression of the lck tyrosine kinase gene in human colon carcinoma and other non-lymphoid human tumor cell lines. Oncogene Res. 1987 Sep-Oct; 1(4):357-74.

Specific Activity



The specific activity of LCK was determined to be **27 nmol /min/mg** as per activity assay protocol, and was equivalent to **180 nmol/min/mg** as per radiometric assay.

Purity



The purity was determined to be **>95%** by densitometry.
Approx. MW **84kDa**.

Full-length human recombinant protein expressed in Sf9 cells

Catalog #	L03-10G
Specific Activity	27 nmol/min/mg
Lot #	C2007-6
Purity	>95%
Concentration	0.1µg/µl
Stability	1yr at –70°C from date of shipment
Storage & Shipping	Store product at –70°C. For optimal storage, aliquot target into smaller quantities after centrifugation and store at recommended temperature. For most favorable performance, avoid repeated handling and multiple freeze/thaw cycles. Product shipped on dry ice.

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Activity Assay Protocol

Reaction Components

Active Kinase (Catalog #: L03-10G)

Active LCK (0.1μg/μl) diluted with Kinase Dilution Buffer X (1x) (Catalog #: K20-09) and assayed as outlined in sample activity plot. (Note: these are suggested working dilutions and it is recommended that the researcher perform a serial dilution of active LCK for optimal results).

ADP-Glo™ Kinase Assay Kit (Promega, Cat # V9101)

ATP solution, 10 mM
ADP solution, 10 mM
ADP-Glo™ Reagent
Kinase Detection Reagent

Kinase Assay Buffer III (5x) (Catalog #: K03-09)

Buffer components: 200mM Tris-HCl, pH 7.4, 100mM MgCl2 and 0.5mg/ml BSA. Add fresh DTT prior to use to a final concentration of 250μM.

Substrate (Catalog #: P61-58)

Poly (4:1 Glu, Tyr) synthetic peptide substrate diluted in distilled H2O to a final concentration of 1mg/ml.

Kinase Dilution Buffer X (1x) (Catalog #: K20-09)

Kinase Assay Buffer III (Catalog #: K03-09) with 12.5mM $MnCl_2$ diluted at a 1:4 ratio (5X dilution) with cold water. Add fresh DTT to the aliquot prior to use to a final concentration of 50μM.

Cofactor: 2.5M $MnCl_2$ (Catalog #: M40-09-25)

Diluted to a working concentration of 0.1M in distilled H2O.

Assay Protocol

The LCK assay is performed using the ADP-Glo™ Kinase Assay kit (Promega; Cat# V9101) which quantifies the amount of ADP produced by the LCK reaction. The ADP- Glo™ Reagent is added to terminate the kinase reaction and to deplete the remaining ATP, and then the Kinase Detection Reagent is added to convert ADP to ATP and to measure the newly synthesized ATP using luciferase/luciferin reaction.

Step 1. Thaw the Active LCK, Kinase Assay Buffer III (5x), and Substrate on ice. Prepare a 15 μL enzyme dilution at the desired concentration, with Kinase Dilution Buffer X (1x), in a pre-chilled 96-well plate.

Step 2. Prepare a substrate/ATP mixture as follows (25 μM example):

Component	Amount (μL)	Component	Amount (μL)
10mM ATP Solution	1.25	Substrate at 1mg/mL	50
Kinase Assay Buffer III (5x)	46.75	0.1M $MnCl_2$	2

Step 3. Transfer the following reaction components prepared in Step 2 to a 384-well opaque plate bringing the reaction volume up to 5μL:

Component 1. 3μl of diluted Active LCK (Catalog # L03-10G).
Component 2. 2μl of Substrate/ATP mix as prepared in the table above. This initiates the reaction.

Step 4. Set up the blank control as outlined in step 2, excluding the addition of the kinase. Replace the kinase with an equal volume of Kinase Dilution Buffer X (1x).

Step 5. Incubate at ambient temperature for 40 minutes.

Step 6. After the 40-minute incubation period, terminate the reaction and deplete the remaining ATP by adding 5μl of ADP-Glo™ Reagent. Spin down and shake the 384-well plate. Then incubate the reaction mixture for another 40 minutes at ambient temperature.

Step 7. Then add 10μl of the Kinase Detection Reagent to the 384-well plate and incubate the reaction mixture for another 30 minutes at ambient temperature.

Step 8. Read the 384-well reaction plate using the Luminescence Module Protocol on a GloMax®-Multi Microplate Multimode Reader (Promega; Cat# E7061).

Step 9. Determine the corrected activity (RLU) by removing the blank control value (see Step 4) for each sample and calculate the kinase specific activity as outlined below.

Calculation of Specific Activity of ADP (RLU/pmol)

From ADP standard curve, determine RLU/pmol of ADP

Kinase Specific Activity (SA) (pmol/min/μg or nmol/min/mg)

Corrected RLU from reaction / [(SA of ADP in RLU/pmol)*(Reaction time in min)*(Enzyme amount in μg or mg)

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MATERIAL SAFETY DATA SHEET

Article 1 - Product Identification and Use

Product Name: LCK, Active **Catalog # L03-10G**

This product is sold only for research use by qualified laboratory personnel, and is not to be used as a drug, medical device, food additive, cosmetic, nor household chemical. It is not to be used in diagnostic, therapeutic, consumer, agricultural, nor pesticidal applications.

Manufacturer's Name:	SignalChem Pharmaceuticals Inc.
Street Address:	*110-13120 Vanier Place*
City, Prov. Postal Code:	*Richmond, BC, V6V 2J2*
Fax:	604-232-4601
EMERGENCY PHONE:	604-232-4600

Article 2 - Hazardous Ingredients

NOT AVAILABLE. We are not aware of any hazards associated with this product or its ingredients, but the chemical, physical, and toxicological properties of this product have not been investigated thoroughly. Observe normal laboratory precautions.

Article 3 - Physical Data

This product consists of purified protein in Tris-HCl buffer shipped on dry ice. The physical properties of this product have not been investigated thoroughly.

Article 4 - Fire and Explosion Hazard

NOT APPLICABLE

Article 5 - Reactivity Data

NOT APPLICABLE

Article 6 – Toxicologically Data

May be harmful by inhalation, ingestion, or skin absorption. The toxicological properties of this product have not been investigated thoroughly. Exercise due caution.

Article 7 - Preventative Measures

Wear chemical safety goggles and compatible chemical-resistant gloves. Avoid inhalation, contact with eyes, skin or clothing.

*****MULTIPLE COMPONENT SPILL OR LEAK PROCEDURES*****

- Wear protective equipment.
- Absorb on sand or vermiculite and place in closed containers for disposal.
- Observe all federal, state and local environmental regulations.

Article 8 - First Aid Measures

- If swallowed, wash out mouth with water, provided person is conscious. Call a physician.
- In case of skin contact, flush with copious amounts of water for at least 15 minutes. Remove contaminated clothing and shoes. If a rash or other irritation develops, call a physician.
- If inhaled, remove to fresh air. If breathing becomes difficult, call a physician.
- In case of eye contact, flush with copious amounts of water for at least 15 minutes while separating the eyelids with fingers. Call a physician.

Article 9 – Preparation

Prepared by: Mya Zhang Phone#: 1-866-954-6273