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Data Sheet
LCK Assay Kit
Catalog #79794
96 Reactions

BACKGROUND: LCK is a tyrosine kinase that phosphorylates the CD3 receptor and is essential for T cell development and activation. LCK also associates with the cytoplasmic domains of the CD4 and CD8 glycoproteins and interacts with the beta-chain of the interleukin-2 receptor. LCK inhibitors are being investigated as a promising therapeutic approach to autoimmune disease and other inflammatory disorders.

DESCRIPTION: The *LCK Assay Kit* is designed to measure LCK activity for screening and profiling applications using Kinase-Glo[®] MAX as a detection reagent. The *LCK Assay Kit* comes in a convenient 96-well format, with enough purified recombinant LCK, Protein Tyrosine Kinase Substrate (Poly-Glu,Tyr 4:1), ATP, and kinase assay buffer for 96 enzyme reactions.

COMPONENTS:

Catalog #	Reagent	Amount	Storage	
40470	LCK, GST-Tag	5 µg	-80°C	Avoid multiple freeze/thaw cycles!
79793	5x Kinase Buffer 2	1.5 ml	-20°C	
79686	ATP (500 µM)	100 µl	-20°C	
40217	Protein Tyrosine Kinase Substrate (Poly-Glu,Tyr 4:1) (10 mg/ml)	100 µl	-20°C	
79696	96-well plate, white	1	RT	

MATERIALS OR INSTRUMENTS REQUIRED BUT NOT SUPPLIED:

Kinase-Glo MAX (Promega #V6071)
Dithiothreitol (DTT, 0.5 M; optional)
Microplate reader capable of reading luminescence
Adjustable micropipettor and sterile tips
30°C incubator

APPLICATIONS: Useful for studying enzyme kinetics and screening small molecular inhibitors for drug discovery and HTS applications.

STABILITY: Up to 6 months when stored as recommended.

REFERENCES:

1. Yu, C.-L., Jove, R., and Burakoff, S.J. 1997. "Constitutive activation of the Janus kinase-STAT pathway in T lymphoma overexpressing the LCK protein tyrosine kinase." *J. Immunology* **159 (11):** 5206-5210.

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2. Veillette, A., *et al.* 1989. "Signal transduction through the CD4 receptor involves the activation of the internal membrane tyrosine-protein kinase p56LCK." *Nature* **338(6212)**: 257-259.

ASSAY PROTOCOL:

All samples and controls should be tested in duplicate.

- 1) Thaw **5x Kinase assay buffer**, **ATP (500 μ M)**, and **Protein Tyrosine Kinase Substrate (Poly-Glu,Tyr 4:1)**.
(Optional: If desired, add 30 μ l of 0.5 M DTT to **5x Kinase assay buffer**).
- 2) Prepare the master mixture (25 μ l per well): N wells x (10 μ l **5x Kinase assay buffer** + 1 μ l **ATP (500 μ M)** + 1 μ l **Protein Tyrosine Kinase Substrate (Poly-Glu,Tyr 4:1)** + 13 μ l distilled water). Add 25 μ l to every well.

	Positive Control	Test Inhibitor	Blank
5x Kinase assay buffer	10 μ l	10 μ l	10 μ l
ATP (500 μ M)	1 μ l	1 μ l	1 μ l
Poly-Glu,Tyr(10 mg/ml)	1 μ l	1 μ l	1 μ l
Water	13 μ l	13 μ l	13 μ l
Test Inhibitor	-	5 μ l	-
Inhibitor buffer	5 μ l	-	5 μ l
1x Kinase buffer	-	-	20 μ l
LCK, GST-tag (1ng/ μ l)	20 μ l	20 μ l	-
Total	50 μl	50 μl	50 μl

- 3) Add 5 μ l of Inhibitor solution of each well labeled as "Test Inhibitor." For the "Positive Control" and "Blank," add 5 μ l of the same solution without inhibitor (Inhibitor buffer).
Note: Final DMSO concentration must be \leq 1%. Higher DMSO levels can significantly decrease the enzyme activity. For example, to test an inhibitor dissolved in 100% DMSO at 10 μ M, dilute 1 mM inhibitor with water to make a 100 μ M inhibitor in 10% DMSO(aq). Then, add 5 μ l of the 100 μ M solution into the 50 μ l assay to make a 1% DMSO concentration in the final reaction mixture. In this example, the inhibitor buffer for the "Positive Control" and "Blank wells" would be 10% DMSO in water.
- 4) Prepare 3 ml of **1x Kinase assay buffer** by mixing 600 μ l of **5x Kinase assay buffer** with 2400 μ l water. 3 ml of **1x Kinase assay buffer** is sufficient for 100 reactions.
- 5) To the wells designated as "Blank," add 20 μ l of **1x Kinase assay buffer**.

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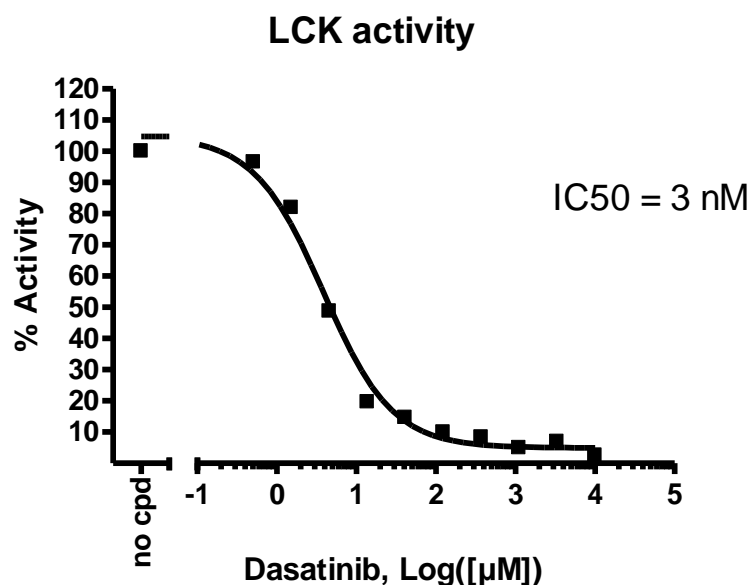
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- 6) Thaw **LCK, GST-Tag** on ice. Upon first thaw, briefly spin tube containing enzyme to recover full content of the tube. Calculate the amount of **LCK, GST-Tag** required for the assay and dilute enzyme to 1 ng/ μ l with **1x Kinase assay buffer**. Store remaining undiluted enzyme in aliquots at -80°C . *Note: LCK, GST-Tag is sensitive to freeze/thaw cycles. Avoid multiple freeze/thaw cycles. Do not re-use thawed aliquots or diluted enzyme.*
- 7) Initiate reaction by adding 20 μ l of diluted **LCK, GST-Tag** to the wells designated "Positive Control" and "Test Inhibitor." Incubate at 30°C for 45 minutes.
- 8) Thaw Kinase-Glo Max reagent.
- 9) After the 45 minute reaction, add 50 μ l of Kinase-Glo Max reagent to each well. Cover plate with aluminum foil and incubate the plate at room temperature for 15 minutes.
- 10) Measure luminescence using the microplate reader. "Blank" value should be subtracted from all wells.

Example of Assay Results:



Inhibition of LCK, GST-Tag by Dasatinib, measured using the LCK assay kit (BPS Bioscience #79794). *Data shown is lot-specific. For lot-specific information, please contact BPS Bioscience, Inc. at info@bpsbioscience.com*

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RELATED PRODUCTS:

<u>Product Name</u>	<u>Catalog #</u>	<u>Size</u>
LCK, GST-tag	40470	10 µg
Kinase Buffer 1	79334	10 ml
Protein Tyrosine Kinase Substrate (poly-Glu,Tyr 4:1)	40217	1 mg
SRC Assay Kit	79680	96 rxns.
YES Assay Kit	79681	96 rxns.
SYK Assay Kit	79671	96 rxns.

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