SZABO SCANDIC

Part of Europa Biosite

Produktinformation

- Forschungsprodukte & Biochemikalien
- Zellkultur & Verbrauchsmaterial
- Diagnostik & molekulare Diagnostik
- Laborgeräte & Service

Weitere Information auf den folgenden Seiten!
See the following pages for more information!

Lieferung & Zahlungsart

siehe unsere Liefer- und Versandbedingungen

Zuschläge

- Mindermengenzuschlag
- Trockeneiszuschlag
- Gefahrgutzuschlag
- Expressversand

SZABO-SCANDIC HandelsgmbH

Quellenstraße 110, A-1100 Wien

T. +43(0)1 489 3961-0

F. +43(0)1 489 3961-7

mail@szabo-scandic.com

www.szabo-scandic.com

linkedin.com/company/szaboscandic

6042 Cornerstone Court W, Ste B
San Diego, CA 92121
Tel: 1.858.202.1401
Fax: 1.858.481.8694
Email: info@bpsbioscience.com

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.

6042 Cornerstone Court W, Ste B
San Diego, CA 92121
Tel: 1.858.202.1401
Fax: 1.858.481.8694
Email: info@bpsbioscience.com

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 µM),** and **Protein Tyrosine Kinase Substrate (Poly-Glu,Tyr 4:1)**.
(Optional: If desired, add 30 µl of 0.5 M DTT to **5x Kinase assay buffer**).

2) Prepare the master mixture (25 µl per well): N wells x (10 µl **5x Kinase assay buffer** + 1 µl **ATP (500 µM)** + 1 µl **Protein Tyrosine Kinase Substrate (Poly-Glu,Tyr 4:1)** + 13 µl distilled water). Add 25 µl to every well.

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

3) Add 5 µl of Inhibitor solution of each well labeled as “Test Inhibitor.” For the “Positive Control" and “Blank,” add 5 µl of the same solution without inhibitor (Inhibitor buffer).
Note: Final DMSO concentration must be ≤1%. Higher DMSO levels can significantly decrease the enzyme activity. For example, to test an inhibitor dissolved in 100% DMSO at 10 µM, dilute 1 mM inhibitor with water to make a 100 µM inhibitor in 10% DMSO(aq). Then, add 5 µl of the 100 µM solution into the 50 µl 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 µl of **5x Kinase assay buffer** with 2400 µl water. 3 ml of **1x Kinase assay buffer** is sufficient for 100 reactions.

5) To the wells designated as "Blank," add 20 µl of **1x Kinase assay buffer**.

6042 Cornerstone Court W, Ste B
San Diego, CA 92121
Tel: 1.858.202.1401
Fax: 1.858.481.8694
Email: info@bpsbioscience.com

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/µl 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.*

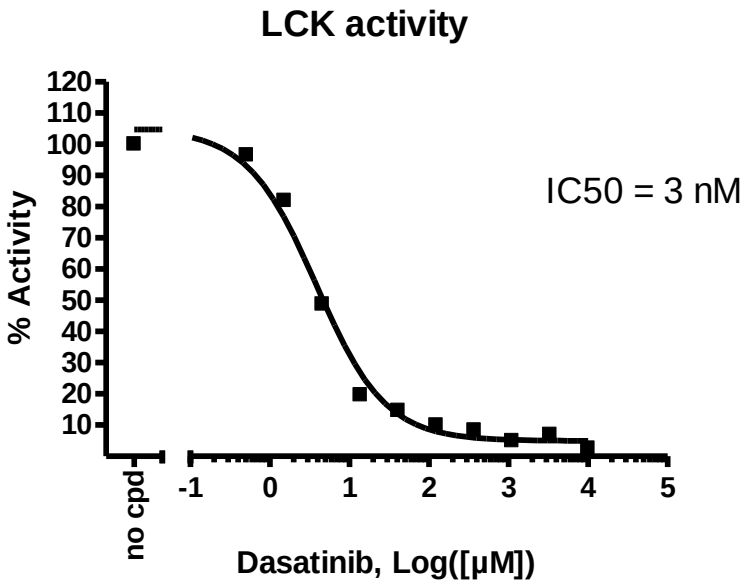
7) Initiate reaction by adding 20 µl of diluted **LCK, GST-Tag** to the wells designated "Positive Control" and "Test Inhibitor." Incubate at 30˚C for 45 minutes.

8) Thaw Kinase-Glo Max reagent.

9) After the 45 minute reaction, add 50 µl 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*

6042 Cornerstone Court W, Ste B
San Diego, CA 92121
Tel: 1.858.202.1401
Fax: 1.858.481.8694
Email: info@bpsbioscience.com

RELATED PRODUCTS:

Product Name	Catalog #	Size
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.