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Data Sheet
SYK Assay Kit
Catalog #79671
96 Reactions

DESCRIPTION: SYK of the nonreceptor tyrosine kinases plays a role in autoimmune diseases like Epstein Barr virus and hematopoietic malignancies. The SYK Assay Kit is designed to measure SYK activity for screening and profiling applications using Kinase-Glo[®] MAX as a detection reagent. The SYK Assay Kit comes in a convenient 96-well format, with enough purified recombinant SYK enzyme, PolyGluTyr peptide, ATP, and kinase assay buffer for 100 enzyme reactions.

COMPONENTS:

Catalog #	Reagent	Amount	Storage	
40487	SYK, Catalytic, GST-tag	2 µg	-80°C	Avoid multiple freeze/thaw cycles!
	5x Kinase assay buffer	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	Room Temp.	

MATERIALS OR INSTRUMENTS REQUIRED BUT NOT SUPPLIED:

Kinase-Glo MAX (Promega #V6071)
Dithiothreitol (DTT, 1 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.

REFERENCE:

1. Furusho, A., Aoki, H., *et al.* Involvement of B Cells, Immunoglobulins, and Syk in the Pathogenesis of Abdominal Aortic Aneurysm. *J. Amer. Heart Assoc.* 2018; **7**:e007750.
2. Law, C.L., Chandran, K.A., *et al.* Phospholipase C-gamma1 interacts with conserved phosphotyrosyl residues in the linker region of Syk and is a substrate for Syk. *Mol. Cell Biol.* 1996; **16(4)**:1305-15.

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ASSAY PROTOCOL:

All samples and controls should be tested in duplicate.

- 1) Thaw **5x Kinase assay buffer**, **ATP (500 μ M)**, and **Poly-Glu,Tyr (10 mg/ml)**.
(Optional: If desired, add DTT to **5x Kinase assay buffer** to make a 10 mM concentration; e.g. add 10 μ l of 1 M DTT to 1 ml **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 **Poly-Glu,Tyr (10 mg/ml)**.+ 13 μ l 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 (no inhibitor)	5 μ l	-	5 μ l
1x Kinase buffer	-	-	20 μ l
SYK (1 ng/ μ l)	20 μ l	20 μ l	-
Total	50 μ l	50 μ l	50 μ l

- 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).
- 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**.
- 6) Thaw **SYK** on ice. Upon first thaw, briefly spin tube containing enzyme to recover full content of the tube. Calculate the amount of **SYK** 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: SYK enzyme 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 **SYK** to the wells designated "Positive Control" and "Test Inhibitor Control." Incubate at 30°C for 45 minutes.

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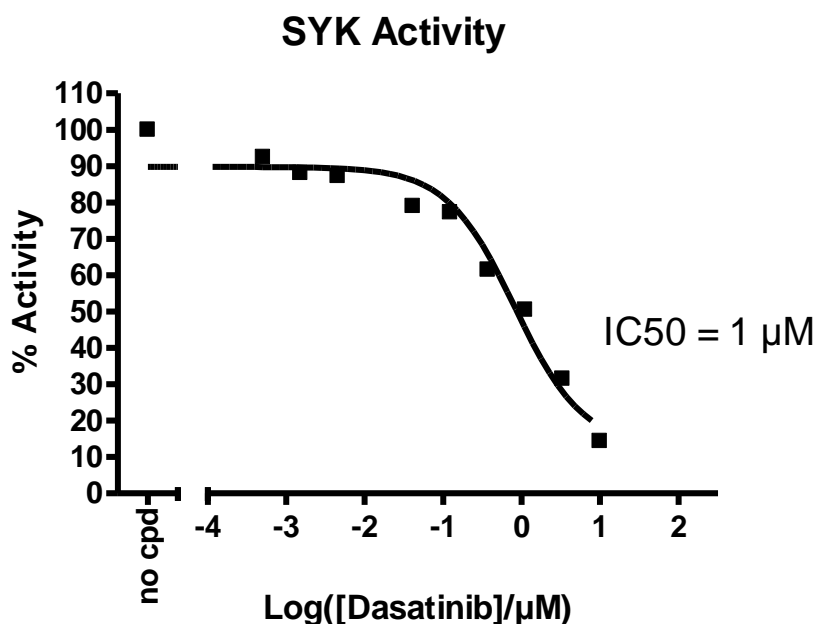
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- 8) Thaw Kinase-Glo Max reagent.
- 9) After the 45 minute reaction, add 50 μ l of Kinase-Glo Max reagent to each well. Cover plate with aluminum foil and incubate the plate at room temperature for 15 minutes.
- 10) Measure luminescence using the microplate reader.

Example of Assay Results:



Inhibition of SYK by Dasatinib, measured using the SYK assay kit (Cat. #79671). *Data shown is lot-specific. For lot-specific information, please contact BPS Bioscience, Inc. at info@bpsbioscience.com*

RELATED PRODUCTS:

<u>Product Name</u>	<u>Catalog #</u>	<u>Size</u>
SYK, Full length, GST-tag	40485	10 μ g
SYK, Catalytic, GST-tag	40487	10 μ g
MALT1, FLAG-Tag (Mouse)	100054	10 μ g
NLRP3 (NALP3), His-FLAG-tags (Sf9)	80117	5 μ g
NLRP3 (K232A), His-FLAG-Tag (Sf9)	40741	10 μ g
ZAP70, GST-tag	40490	10 μ g

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