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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<sup>®</sup> 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	<b>Avoid multiple freeze/thaw cycles!</b>
	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  $\mu$ 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  $\mu$ l of 1 M DTT to 1 ml **5x Kinase assay buffer**)
- 2) Prepare the master mixture (25  $\mu$ l per well): N wells x (10  $\mu$ l **5x Kinase assay buffer** + 1  $\mu$ l **ATP (500  $\mu$ M)** + 1  $\mu$ l **Poly-Glu,Tyr (10 mg/ml)**.+ 13  $\mu$ l water). Add 25  $\mu$ l to every well.

	Positive Control	Test Inhibitor	Blank
5x Kinase assay buffer	10 $\mu$ l	10 $\mu$ l	10 $\mu$ l
ATP (500 $\mu$ M)	1 $\mu$ l	1 $\mu$ l	1 $\mu$ l
Poly-Glu,Tyr(10 mg/ml)	1 $\mu$ l	1 $\mu$ l	1 $\mu$ l
Water	13 $\mu$ l	13 $\mu$ l	13 $\mu$ l
Test Inhibitor	-	5 $\mu$ l	-
Inhibitor Buffer (no inhibitor)	5 $\mu$ l	-	5 $\mu$ l
1x Kinase buffer	-	-	20 $\mu$ l
SYK (1 ng/ $\mu$ l)	20 $\mu$ l	20 $\mu$ l	-
Total	50 $\mu$ l	50 $\mu$ l	50 $\mu$ l

- 3) Add 5  $\mu$ l of Inhibitor solution of each well labeled as "Test Inhibitor." For the "Positive Control" and "Blank," add 5  $\mu$ l of the same solution without inhibitor (Inhibitor buffer).
- 4) Prepare 3 ml of **1x Kinase assay buffer** by mixing 600  $\mu$ l of **5x Kinase assay buffer** with 2400  $\mu$ l water. 3 ml of **1x Kinase assay buffer** is sufficient for 100 reactions.
- 5) To the wells designated as "Blank," add 20  $\mu$ 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/ $\mu$ 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  $\mu$ 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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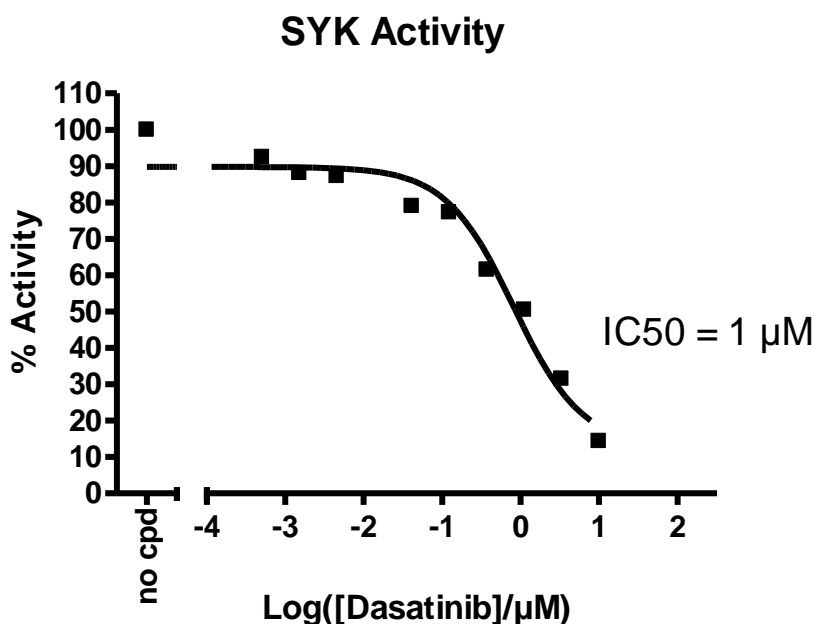
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- 8) Thaw Kinase-Glo Max reagent.
- 9) After the 45 minute reaction, add 50  $\mu$ l of Kinase-Glo Max reagent to each well. Cover plate with aluminum foil and incubate the plate at room temperature for 15 minutes.
- 10) Measure luminescence using 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 $\mu$ g
SYK, Catalytic, GST-tag	40487	10 $\mu$ g
MALT1, FLAG-Tag (Mouse)	100054	10 $\mu$ g
NLRP3 (NALP3), His-FLAG-tags (Sf9)	80117	5 $\mu$ g
NLRP3 (K232A), His-FLAG-Tag (Sf9)	40741	10 $\mu$ g
ZAP70, GST-tag	40490	10 $\mu$ g

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