

Data Sheet SRC Assay Kit Catalog #79680 96 Reactions

DESCRIPTION: SRC is a member of the nonreceptor tyrosine kinases that plays a role in many cellular functions, including cell adhesion, growth, and differentiation. SRC has been implicated in diseases such as chronic kidney disease and metastatic bone disease. The *SRC Assay Kit* is designed to measure SRC activity for screening and profiling applications using Kinase-Glo[®] MAX as a detection reagent. The *SRC Assay Kit* comes in a convenient 96-well format, with enough purified recombinant SRC enzyme, Protein Tyrosine Kinase Substrate (Poly-Glu,Tyr 4:1), ATP, and kinase assay buffer for 100 enzyme reactions.

COMPONENTS:

Catalog #	Reagent	Amount	Storag	ge
40484	SRC, His-tag	>1 µg	-80°C	Avoid
79334	5x Kinase assay buffer	1.5 ml	-20°C	multiple
79686	ATP (500 μM)	100 µl	-20°C	freeze/
40217	Protein Tyrosine Kinase Substrate (Poly-Glu,Tyr 4:1) (10 mg/ml)	100 µl	-20°C	thaw cycles!
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. Vidal, M., *et al.* Differing Src signaling levels have distinct outcomes in Drosophila; 2007, *Cancer Res.* **67(21):** 10278—10285.
- 2. Wan, J. and Zhuang, S. Src Family Kinases in Chronic Kidney Disease; 2017, *Amer. J. Physiol. Renal Physiol.***313(3):** F721–F728.

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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 DTT to **5x Kinase assay buffer** to make a 10 mM concentration; *e.g.* add 10 µl of 1 M DTT to 1 ml **5x Kinase assay buffer**)

 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 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 µI	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 (no inhibitor)	-	-	20 µl
SRC (1.5 ng/µl)	20 µl	20 µl	_
Total	50 µl	50 µl	50 µl

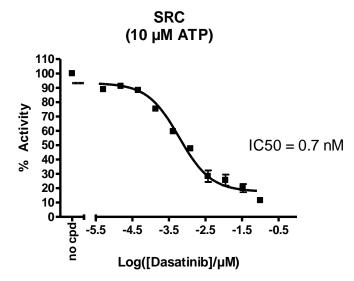
- Add 5 µl of Inhibitor solution of each well labeled as "Test Inhibitor." For the "Positive Control" and "Blank," add 5 µl of the same solution without inhibitor (Inhibitor buffer).
- 4) Prepare 3 ml of **1x Kinase assay buffer** by mixing 600 μl of **5x Kinase assay buffer** with 2400 μl water. 3 ml 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**.
- 6) Thaw SRC on ice. Upon first thaw, briefly spin tube containing enzyme to recover full content of the tube. Calculate the amount of SRC 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. <u>Note</u>: SRC enzyme is sensitive to freeze/thaw cycles. Avoid multiple freeze/thaw cycles. Do not re-use thawed aliquots or diluted enzyme.

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- Initiate reaction by adding 20 µl of diluted SRC to the wells designated "Positive Control" and "Test Inhibitor Control." Incubate at 30°C for 45 minutes.
- 8) Thaw Kinase-Glo Max reagent.
- 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.

Example of Assay Results:



Inhibition of SRC by Dasatinib, measured using the SRC assay kit (BPS Bioscience #79680). Data shown is lot-specific. For lot-specific information, please contact BPS Bioscience, Inc. at info@bpsbioscience.com

RELATED PRODUCTS:					
Catalog #	<u>Size</u>				
40483	10 µg				
40484	10 µg				
40488	10 µg				
40410	10 µg				
40470	10 µg				
40217	1 mg				
	40483 40484 40488 40410 40470				

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