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**Data Sheet**  
***FYN Assay Kit***  
**Catalog #78003**  
**96 Reactions**

**BACKGROUND:** FYN is member of the SRC tyrosine kinase oncogene family. It plays a role in various forms of cancer and is a notable molecular target in prostate cancer. Fyn interacts with both amyloid-beta (A $\beta$ ) and tau, suggesting that inhibition of FYN has potential as a therapy for Alzheimer's disease.

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

**COMPONENTS:**

Catalog #	Reagent	Amount	Storage	
40433	FYN, FLAG-Tag	2 $\mu$ g	-80°C	<b>Avoid multiple freeze/ thaw cycles!</b>
79334	5x Kinase assay buffer	1.5 ml	-20°C	
79686	ATP (500 $\mu$ M)	100 $\mu$ l	-20°C	
40217	Protein Tyrosine Kinase Substrate (Poly-Glu,Tyr 4:1) (10 mg/ml)	100 $\mu$ l	-20°C	
79696	96-well plate, white	1	RT	

**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(S):**

1. Resh, M.D. "Fyn, a Src family tyrosine kinase." *Int. J. Biochem. Cell. Biol.* **30.11** (1998): 1159-1162.
2. Umemori, H., et al. "Initial events of myelination involve Fyn tyrosine kinase signaling." *Nature* **367.6463** (1994): 572-576.

**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

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# **ASSAY PROTOCOL:**

***All samples and controls should be tested in duplicate.***

Thaw **5x Kinase assay buffer**, **ATP (500  $\mu$ M)**, and **Protein Tyrosine Kinase Substrate (Poly-Glu,Tyr 4:1)**. (Optional: If desired, add 30  $\mu$ l of 0.5 M DTT to **5x Kinase assay buffer**).

- 1) 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 **Protein Tyrosine Kinase Substrate (Poly-Glu,Tyr 4:1)** + 13  $\mu$ l distilled 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 (10% DMSO in water)	5 $\mu$ l	–	5 $\mu$ l
1x Kinase buffer	–	–	20 $\mu$ l
FYN, FLAG-Tag (1 ng/ $\mu$ l)	20 $\mu$ l	20 $\mu$ l	–
Total	50 $\mu$ l	50 $\mu$ l	50 $\mu$ l

- 2) 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 (usually 10% DMSO in water). *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  $\mu$ M, dilute 1 mM inhibitor with water to make a 100  $\mu$ M inhibitor in 10% DMSO(aq). Then, add 5  $\mu$ l of the 100  $\mu$ M solution into the 50  $\mu$ l assay to make a 1% DMSO concentration in the final reaction mixture.*

- 3) 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.

- 4) To the wells designated as "Blank," add 20  $\mu$ l of **1x Kinase assay buffer**.

- 5) Thaw **FYN, FLAG-Tag** on ice. Upon first thaw, briefly spin tube containing material to recover full content of the tube. Calculate the amount of **FYN, FLAG-Tag** required for the assay and dilute enzyme to 1 ng/ $\mu$ l with **1x Kinase assay**

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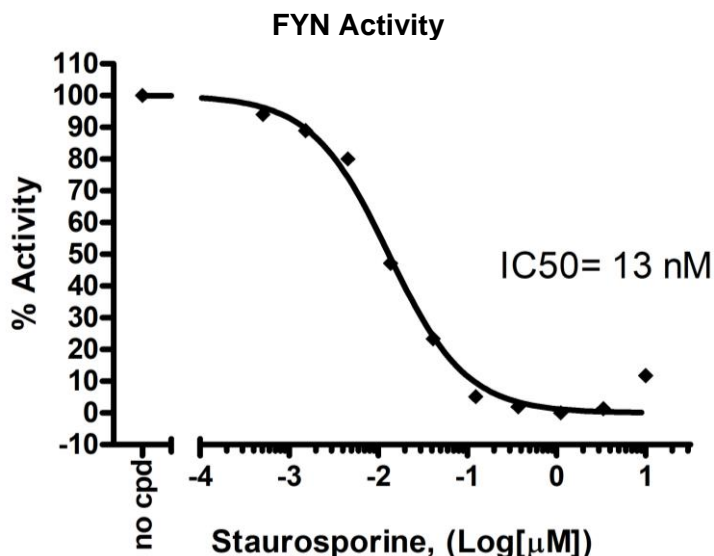
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**buffer.** Store remaining undiluted material in aliquots at -80°C. *Note: FYN, FLAG-Tag is sensitive to freeze/thaw cycles. Avoid multiple freeze/thaw cycles. Do not re-use thawed aliquots or diluted material.*

- 6) Initiate reaction by adding 20 µl of diluted **FYN, FLAG-Tag** to the wells designated "Positive Control" and "Test Inhibitor Control." Incubate at 30°C for 45 minutes.
- 7) Thaw Kinase-Glo Max reagent.
- 8) 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.
- 9) Measure luminescence using the microplate reader. Value of "Blank" reading should be subtracted from all other measurements.

#### Example of Assay Results:



Inhibition of FYN, His-Tag by Staurosporine, measured using the FYN assay kit (BPS Bioscience #78003). *Data shown is lot-specific. For lot-specific information, please contact BPS Bioscience, Inc. at [info@bpsbioscience.com](mailto:info@bpsbioscience.com)*

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**RELATED PRODUCTS:**

<b><u>Product Name</u></b>	<b><u>Catalog #</u></b>	<b><u>Size</u></b>
FYN, FLAG-tag	40433	10 µg
5x Kinase assay buffer	79334	10 ml
ATP (500 µM)	79686	200 µl
Protein Tyrosine Kinase Substrate (poly-Glu,Tyr 4:1)	40217	1 mg
SRC, GST-tag	40483	10 µg
SRC, His-tag	40484	10 µg
LCK, GST-tag	40470	10 µg
FGR, GST-tag	40422	10 µg
YES1, GST-tag	40488	10 µg
SRC Assay Kit	79680	96 rxns.
LCK Assay Kit	79794	96 rxns.
YES1 Assay Kit	79681	96 rxns.

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