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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 β) 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[®] 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 μ g	-80°C	Avoid multiple freeze/thaw cycles!
79334	5x Kinase assay buffer	1.5 ml	-20°C	
79686	ATP (500 μ M)	100 μ l	-20°C	
40217	Protein Tyrosine Kinase Substrate (Poly-Glu,Tyr 4:1) (10 mg/ml)	100 μ 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 μ M)**, and **Protein Tyrosine Kinase Substrate (Poly-Glu,Tyr 4:1)**. (Optional: If desired, add 30 μ l of 0.5 M DTT to **5x Kinase assay buffer**).

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

- 2) 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 (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 μ M, dilute 1 mM inhibitor with water to make a 100 μ M inhibitor in 10% DMSO(aq). Then, add 5 μ l of the 100 μ M solution into the 50 μ 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 μ l of **5x Kinase assay buffer** with 2400 μ l water. 3 ml of **1x Kinase assay buffer** is sufficient for 100 reactions.
- 4) To the wells designated as "Blank," add 20 μ 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/ μ l with **1x Kinase assay**

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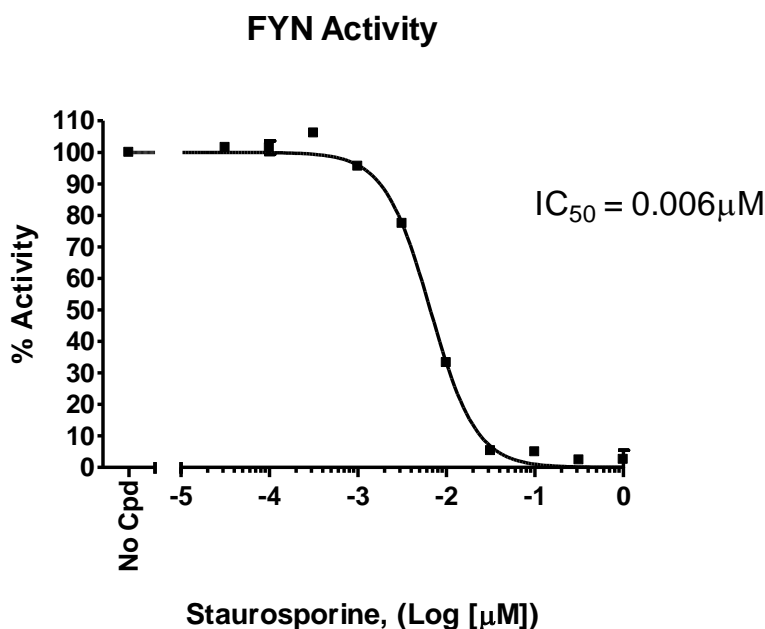
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buffer. Store remaining undiluted material in aliquots at -80°C . *Note: FYN, FLAG-Tag is sensitive to freeze/thaw cycles. Avoid multiple freeze/thaw cycles. Do not re-use thawed aliquots or diluted material.*

- 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 support@bpsbioscience.com*

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RELATED PRODUCTS:

<u>Product Name</u>	<u>Catalog #</u>	<u>Size</u>
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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