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Data Sheet

YES1 Assay Kit

Catalog #79681
96 Reactions

DESCRIPTION: Proto-oncogene tyrosine-protein kinase YES1 has been implicated in regulation of cell growth and survival, apoptosis, cell-cell adhesion, and differentiation. It has been identified as a potential target in basal-like breast cancers. The YES1 Assay Kit is designed to measure YES1 activity for screening and profiling applications using Kinase-Glo® MAX as a detection reagent. The YES1 Assay Kit comes in a convenient 96-well format, with enough purified recombinant YES1 enzyme, Protein Tyrosine Kinase Substrate (Poly-Glu,Tyr 4:1), ATP, and kinase assay buffer for 100 enzyme reactions.

COMPONENTS:

Catalog #	Reagent	Amount	Storage	
40488	YES1, GST-tag	10 µg	-80°C	***Avoid multiple freeze/ thaw cycles!***
79334	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)
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.

REFERENCES:
1. Hamamura K., Tsuji M., *et al.* Functional Activation of Src Family Kinase Yes Protein is Essential for the Enhanced Malignant Properties of Human Melanoma Cells Expressing Ganglioside GD3; 2011, *J.] Biol. Chem.]* **286:** 18526-18537.
2. Bilal, E., Alexe G., *et al.* Identification of the YES1 Kinase as a Therapeutic Target in Basal-Like Breast Cancers; 2010, *Genes & Cancer* Oct; **1(10):** 1063–1073.

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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)**.
2) 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**
3) 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 µl	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 (no inhibitor)	5 µl	–	5 µl
1x Kinase buffer	–	–	20 µl
YES1 (5 ng/µl)	20 µl	20 µl	–
Total	50 µl	50 µl	50 µl

4) 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).

5) 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.

6) To the wells designated as "Blank," add 20 µl of **1x Kinase assay buffer**.

7) Thaw **YES1** on ice. Upon first thaw, briefly spin tube containing enzyme to recover full content of the tube. Calculate the amount of **YES1** 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. *Note: YES1 is sensitive to freeze/thaw cycles. Avoid multiple freeze/thaw cycles. Do not re-use thawed aliquots or diluted enzyme.*

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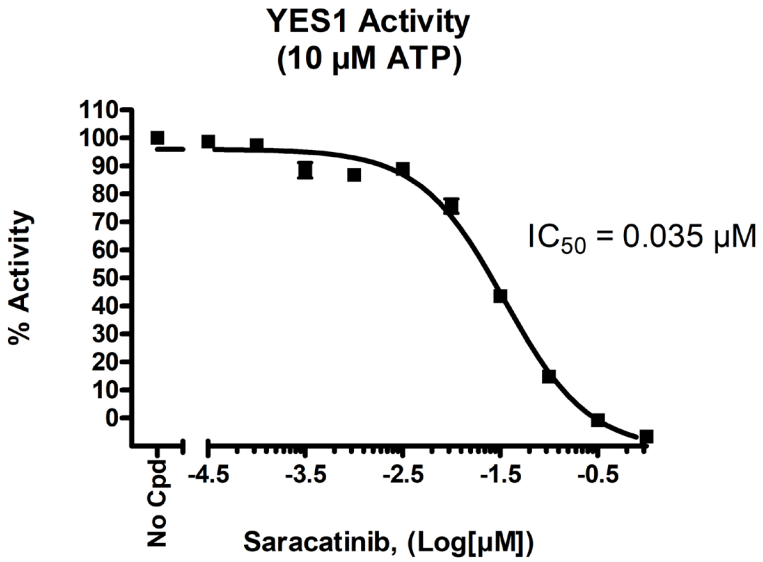
8) Initiate reaction by adding 20 µl of diluted **YES1** to the wells designated “Positive Control” and "Test Inhibitor Control." Incubate at 30˚C for 45 minutes.

9) Thaw Kinase-Glo Max reagent.

10) 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.

11) Measure luminescence using the microplate reader.

Example of Assay Results:



Inhibition of YES1 by Saracatinib, measured using the YES1 assay kit (BPS Bioscience #79681). *Data shown is lot-specific. For lot-specific information, please contact BPS Bioscience, Inc. at info@bpsbioscience.com*

RELATED PRODUCTS:

Product Name	Catalog #	Size
Yes1, GST-tag	40488	10 µg
SRC, GST-tag	40483	10 µg
SRC, His-tag	40484	10 µg
CSK, GST-tag	40410	10 µg
LCK, GST-tag	40470	10 µg
Protein Tyrosine Kinase Substrate (poly-Glu,Tyr 4:1)	40217	1 mg

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