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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; 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.

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 μ M)**, 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 μ l of 1 M DTT to 1 ml **5x Kinase assay buffer**)

- 2) 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 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 (no inhibitor)	5 μ l	-	5 μ l
1x Kinase buffer	-	-	20 μ l
YES1 (5 ng/ μ l)	20 μ l	20 μ l	-
Total	50 μl	50 μl	50 μl

- 3) 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 (Inhibitor buffer).
- 4) 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.
- 5) To the wells designated as "Blank," add 20 μ l of **1x Kinase assay buffer**.
- 6) 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/ μ l 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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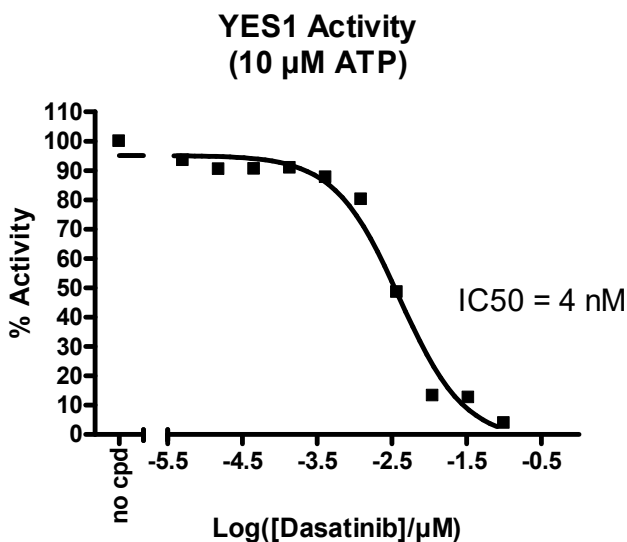
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- 7) Initiate reaction by adding 20 μ l of diluted **YES1** to the wells designated "Positive Control" and "Test Inhibitor Control." Incubate at 30°C for 45 minutes.
- 8) Thaw Kinase-Glo Max reagent.
- 9) After the 45 minute reaction, add 50 μ l of 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 YES1 by Dasatinib, 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:

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

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