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Produktinformation

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- Diagnostik & molekulare Diagnostik
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Lieferung & Zahlungsart

siehe unsere Liefer- und Versandbedingungen

Zuschläge

- Mindermengenzuschlag
- Trockeneiszuschlag
- Gefahrgutzuschlag
- Expressversand

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Data Sheet

Wee1 Assay Kit

Catalog #79909
96 Reactions

BACKGROUND: Wee1 is a tyrosine kinase that is overexpressed in many cancer types such as luminal and HER2-positive breast cancer subtypes, hepatocellular carcinomas, and glioblastomas. Wee1 plays a crucial role in regulating the cell division cycle through phosphorylation of CDKs. Inhibiting Wee1 has been shown to prevent the cells from repairing DNA damage due to unchecked replication, suggesting possible combination therapeutics of the DNA damaging reagents and Wee1 inhibitors.

DESCRIPTION: The *Wee1 Assay Kit* is designed to measure Wee1 activity for screening and profiling applications using Kinase-Glo® Kinase Assay as a detection reagent. The *Wee1 Assay Kit* comes in a convenient 96-well format, with enough purified recombinant Wee1 enzyme, Wee1 Substrate, ATP, and kinase assay buffer for 100 enzyme reactions.

COMPONENTS:

Catalog #	Reagent	Amount	Storage	
40412	Wee1	15 µg	-80°C	***Avoid multiple freeze/ thaw cycles!***
79334	5x Kinase assay buffer 1	1.5 ml	-20°C	
79686	ATP (500 µM)	100 µl	-20°C	
79910	Wee1 Substrate (Poly-Lys,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® Kinase Assay (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. Parker, L., *et al., Science* 1992; **257(5078):**1955.
2. Lundgreen, K., *et al., Cell* 1991 Mar; **64(6):**1111-1122.

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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 **Wee1 Substrate (Poly-Lys,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**)

2) Prepare the master mixture (25 µl per well): N wells x (6 µl **5x Kinase assay buffer** + 1 µl **ATP (500 µM)** + 1 µl **Wee1 Substrate (Poly-Lys,Tyr 4:1)** + 17 µl distilled water. Add 25 µl to every well.

	Positive Control	Test Inhibitor	Blank
5x Kinase assay buffer	6 µl	6 µl	6 µl
ATP (500 µM)	1 µl	1 µl	1 µl
Wee1 Substrate (Poly-Lys,Tyr 4:1)	1 µl	1 µl	1 µl
Water	17 µl	17 µl	17 µl
Test Inhibitor	–	5 µl	–
Inhibitor Buffer (no inhibitor)	5 µl	–	5 µl
1x Kinase buffer	–	–	20 µl
Wee1 (~6 ng/µl)	20 µl	20 µl	–
Total	50 µl	50 µl	50 µl

1) Prepare 10X concentrated inhibitor in an aqueous-based solution. *Note: Final DMSO concentration must be ≤1%. Higher DMSO levels can significantly decrease the enzyme activity. For example, to test an inhibitor dissolved in 100% DMSO at 10 µM, dilute 1 mM inhibitor with water to make a 100 µM inhibitor in 10% DMSO(aq). Then, add 5 µl of the 100 µM solution to the assay to make a 1% DMSO concentration in the final reaction mixture.*

3) 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). Be sure to maintain the same concentration of DMSO as the test sample, e.g. 10% DMSO(aq) for the example above.

2) Prepare 3 ml of **1x Kinase assay buffer** by mixing 600 µl of **5x Kinase assay buffer** with 2400 µl distilled water. 3 ml of **1x Kinase assay buffer** is sufficient for 100 reactions.

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

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4) Thaw **Wee1** on ice. Upon first thaw, briefly spin tube containing enzyme to recover full content of the tube. Calculate the amount of **Wee1** required for the assay and dilute enzyme to 6 ng/µl with **1x Kinase assay buffer**. Store remaining undiluted enzyme in aliquots at -80°C.

   Note: Wee1 enzyme is sensitive to freeze/thaw cycles. Avoid multiple freeze/thaw cycles. Do not re-use thawed aliquots or diluted enzyme.

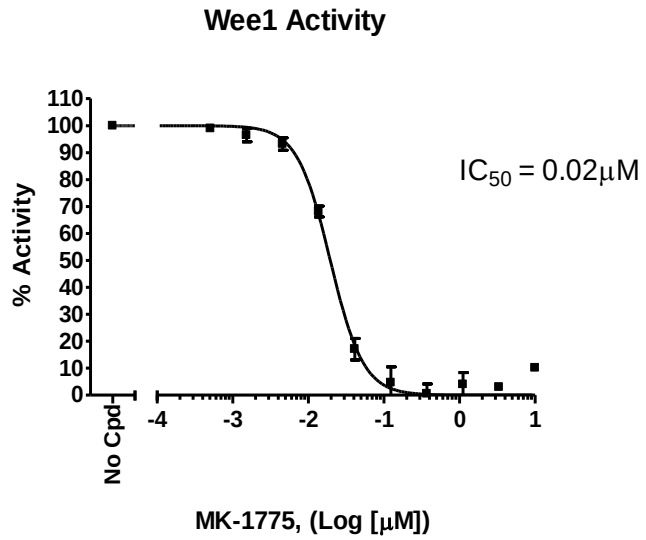
5) Initiate reaction by adding 20 µl of diluted **Wee1** to the wells designated "Positive Control" and "Test Inhibitor Control." Incubate at 30°C for 45 minutes.

6) Thaw Kinase-Glo Max reagent.

7) 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.

8) Measure luminescence using the microplate reader. Value of "Blank" reading should be subtracted from all other measurements.

Example of Assay Results:



Inhibition of Wee1 by MK-1775, measured using the *Wee1 assay kit* (BPS Bioscience #79909). *Data shown is lot-specific. For lot-specific information, please contact BPS Bioscience, Inc. at info@bpsbioscience.com*

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RELATED PRODUCTS:

Product Name	Catalog #	Size
Wee1, Gst-Th-tag	40412	10 µg
5x Kinase buffer 1	79334	10 ml
ATP (500 µM)	79686	200 µl
CDK1/CyclinA2, GST-tag	40100	10 µg
CDK1/CyclinB1, GST-tag	40454	10 µg
CDK1 Assay Kit	79597	96 rxns.

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