Wee1 Kinase Assay Kit

#79909
96 reactions

Description

The Wee1 Kinase Assay Kit is designed to measure Wee1 kinase activity for screening and profiling applications using Kinase-Glo® Kinase Assay as a detection reagent. The Wee1 Kinase Assay Kit comes in a convenient 96-well format, with enough purified recombinant Wee1 enzyme (amino acids 215-646), Wee1 Substrate, ATP, and kinase assay buffer for 100 enzyme reactions.

Background

Wee1 (also known as WEE1 G2 checkpoint kinase) belongs to the serine-threonine kinase family of proteins. Wee1 plays a crucial role in regulating G2/M transition through phosphorylation of cyclin dependent kinase (CDKs), allowing DNA damage to be repaired. that is overexpressed in many types of cancer such as luminal and HER2-positive breast cancer, hepatocellular carcinomas, and glioblastomas. Classical cancer therapies, such as chemotherapy and radiotherapy, induce DNA damage, and cells trigger the checkpoint in cell cycle, such as Wee1. This can lead to cancer cell resistance. Inhibiting Wee1 prevents cells from repairing DNA damage due to unchecked replication, suggesting that combination therapeutics with DNA damaging reagents and Wee1 inhibitors may be effective against cancer.

Application(s)

Study enzyme kinetics and screen small molecule inhibitors for drug discovery and high throughput screening (HTS) applications.

Supplied Materials

Catalog #	Name	Amount	Storage
40412	Wee1, GST-Th-Tag*	15 µg	-80°C
79334	5x Kinase Assay Buffer 1	1.5 ml	-20°C
79686	500 µM ATP	100 µl	-20°C
101574	Wee1 Substrate (5 mg/ml)	200 µl	-80°C
79696	White 96-well plate	1	Room Temperature

**The concentration of the protein is lot-specific and will be indicated on the tube.*

Materials Required but Not Supplied

Name	Ordering Information
Kinase-Glo® Kinase Assay	Promega #V6071
DTT (Dithiothreitol), 1M, optional	
Microplate reader capable of reading luminescence	
Adjustable micropipettor and sterile tips	
30°C incubator	

Storage Conditions

This assay kit will perform optimally for up to **6 months** from date of receipt when the materials are stored as directed.

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• bpsbioscience.com • 858-202-1401 • support@bpsbioscience.com

Safety

This product is for research purposes only and not for human or therapeutic use. This product should be considered hazardous and is harmful by inhalation, in contact with skin, eyes, clothing, and if swallowed. If contact occurs, wash thoroughly.

Assay Principle

Kinase activity is measured using **Kinase-Glo™ Max** (Promega #V6071). The addition of the reagent results in the generation of a luminescent signal that correlates with the amount of ATP. The reagent is linear to 100 µM ATP.

Contraindications

The final concentration of DMSO in the assay should not exceed 1%.

Assay Protocol

- All samples and controls should be tested in duplicate.
- The assay should include "Blank", "Positive Control" and "Test Inhibitor" conditions.
- We recommend maintaining the diluted protein on ice during use.
- For detailed information on protein handling please refer to Protein FAQs (bpsbioscience.com).
- We recommend using MK-1775 (#82196) as internal control. If not running a dose response curve for the control inhibitor, we recommend running the control inhibitor at 0.1X, 1X and 10X the IC_{50} value shown in the validation data below.

1. Thaw **5x Kinase Assay Buffer**, **500 µM ATP,** and **Wee1 Substrate (5 mg/ml).**

   Optional: *If desired, make **5x Kinase Assay Buffer 1** with 10 mM DTT.*

2. Prepare a Master Mix (25 µl/well): N wells x (6 µl of **5x Kinase Assay Buffer** + 1 µl of **500 µM ATP**+ 2 µl of **Wee1 Substrate (5 mg/ml)** + 16 µl of distilled water).

3. Add 25 µl to every well.

4. Prepare 3 ml of **1x Kinase Assay Buffer** by mixing 600 µl of **5x Kinase Assay Buffer** with 2400 µl of distilled water.

   *Note: Three (3) ml of **1x Kinase Assay Buffer** is sufficient for 100 reactions.*

5. Prepare the Test Inhibitor (5 µl/well): for a titration, prepare serial dilutions at concentrations 10-fold higher than the desired final concentrations. The final volume of the reaction is 50 µl.

   5.1 If the Test Inhibitor is water-soluble, prepare serial dilutions in the 1x Kinase Assay Buffer, 10-fold more concentrated than the desired final concentrations.

   For the positive and negative controls, use 1x Kinase Assay Buffer (Diluent Solution).

OR

5.2 If the Test inhibitor is soluble in DMSO, prepare the test inhibitor at 100-fold the highest desired concentration in 100% DMSO, then dilute the inhibitor 10-fold in 1x Kinase Assay Buffer to prepare the highest concentration of the 10-fold intermediate dilutions. The concentration of DMSO is now 10%.

Prepare serial dilutions of the Test Inhibitor at 10-fold the desired final concentrations using 10% DMSO in 1x Kinase Assay Buffer to keep the concentration of DMSO constant.

For positive and negative controls, prepare 10% DMSO in water (vol/vol) so that all wells contain the same amount of DMSO (Diluent Solution).

Note: The final concentration of DMSO should not exceed 1%.

6. Add 5 µl of Test Inhibitor to wells labeled as “Test Inhibitor”.
7. Add 5 µl of the Diluent Solution to the “Positive Control" and “Blank” wells.
8. Add 20 µl of **1x Kinase Assay Buffer** to the “Blank” wells.
9. Thaw **Wee1** on ice. Briefly spin the tube to recover its full content.
10. Dilute the enzyme (20 µl/well) to 6 ng/µl with **1x Kinase Assay Buffer**.
11. Initiate the reaction by adding 20 µl of diluted **Wee1** to the wells designated “Positive Control” and "Test Inhibitor Control".

Component	Blank	Positive Control	Test Inhibitor
Master Mix	25 µl	25 µl	25 µl
Test Inhibitor	-	-	5 µl
Diluent Solution	5 µl	5 µl	-
1x Kinase Assay Buffer	20 µl	-	-
Diluted Wee1 (6 ng/µl)	-	20 µl	20 µl
Total	**50 µl**	**50 µl**	**50 µl**

12. Incubate at 30°C for 45 minutes.
13. Thaw the Kinase-Glo Max™ reagent.
14. At the end of the 45 minute reaction, add 50 µl of Kinase-Glo Max™ reagent to each well.
15. Cover the plate with aluminum foil and incubate at Room Temperature for 15 minutes.
16. Measure luminescence using the microplate reader.
17. The value of “Blank” reading should be subtracted from all other measurements.

Example of Assay Results

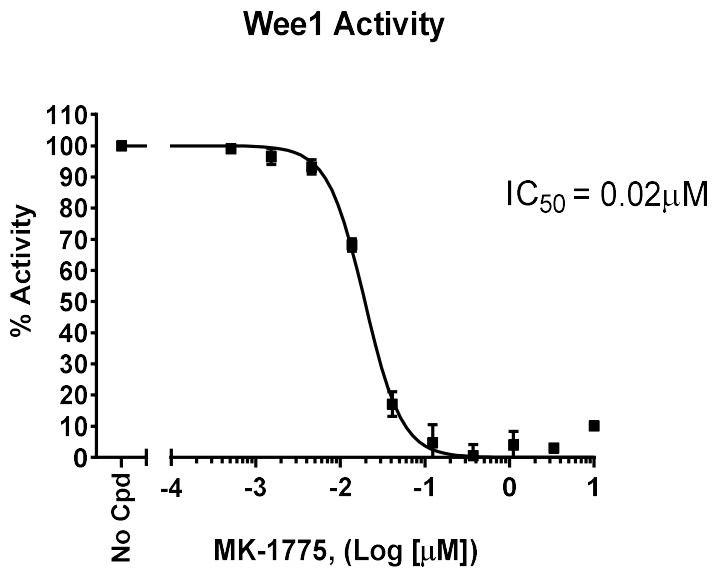

Figure 1. Inhibition of Wee1 by the inhibitor MK-1775.
Wee1 kinase activity was measured in the presence of increasing concentrations of MK-1775 (also known as adavosertib, #82196). The "Blank" value was subtracted from all other values. Results are expressed as the percent of control (kinase activity in the absence of inhibitor, set at 100%).

Data are representative. For lot-specific information, please contact BPS Bioscience, Inc. at info@bpsbioscience.com

References

Parker L., *et al*., *1992 Science,* 257(5078): 1955.
Lundgreen K., *et al.,* 1991 *Cell*, 64(6): 1111-1122.
Vakili-Samiani S., *et al.*, 2022 *Mutat Res* 824: 111776.

Related Products

Products	*Catalog #*	*Size*
MK-1775	82196	10 mg
Wee1, FLAG-Tag Recombinant	100154	10 µg
CDK1 Assay Kit	79597	96 reactions
CDK1/CyclinB1 Kinase Assay Kit	79628	96 reactions/384 reactions
PROTAC® Optimization Kit for CDK Kinase/Cereblon Binding	79924	384 reactions
CRISPR/Cas9 Kinase Knockout Lentivirus Library (Array Format)	78487	200 µl x 649

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