**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.

**Background**

Wee1 (also known as WEE1 G2 checkpoint kinase) 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 cell division through phosphorylation of cyclin dependent kinase (CDKs). 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 molecular inhibitors for drug discovery and high throughput (HTS) applications.

**Supplied Materials**

| Catalog # | Name | Amount | Storage |
|---|---|---|---|
| 40412 | Wee1* | 15 µg | -80°C |
| 79334 | 5x Kinase assay buffer 1 | 1.5 ml | -20°C |
| 79686 | ATP (500 µM) | 100 µl | -20°C |
| | 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.

**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.

1) Thaw **5x Kinase Assay Buffer**, **ATP (500 µM),** and **Wee1 Substrate (5 mg/ml)**
   Optional: If desired, add DTT to **5x Kinase Assay Buffer** to prepare a 10 mM DDT concentration. For example, add 10 µl of 1 M DTT to 1 ml **5x Kinase Assay Buffer**.

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

3) Prepare 3 ml of **1x Kinase Assay Buffer** by mixing 600 µl of **5x Kinase Assay Buffer** with 2400 µl distilled water. Three (3) ml of **1x Kinase Assay Buffer** is sufficient for 100 reactions.

4) 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.

   i) 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).

   ii) If the Test inhibitor is soluble in DMSO, prepare the test inhibitor at 100-fold the highest desired concentration in 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).

   The final concentration of DMSO should not exceed 1%.

5) Add 5 µl of Test Inhibitor to wells labeled as “Test Inhibitor.” For the “Positive Control" and “Blank,” add 5 µl of the Diluent Solution (either kinase assay buffer or 10% DMSO in kinase assay buffer, as described above).

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

7) Thaw **Wee1** on ice. Briefly spin the tube to recover its full contents. Calculate the amount of protein kinase required for the assay and dilute enough for the assay. Aliquot unused protein into 2-4 aliquots as may be necessary and store them at -80°C.

   *Note: The concentration of protein is lot-specific and is indicated on the tube. Verify the initial concentration and dilute accordingly.*

8) Dilute the enzyme (20 µl/well) to 6 ng/µl with **1x Kinase Assay Buffer**. Store the remaining undiluted enzyme in aliquots at -80°C.

   *Avoid multiple freeze/thaw cycles. Do not re-use the aliquots more than once or twice and do not re-use the diluted kinase.*

9) 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 (no inhibitor) | 5 µl | 5 µl | - |
| 1x Kinase Assay Buffer | 20 µl | - | - |
| Wee1 (6 ng/µl) | - | 20 µl | 20 µl |
| **Total** | **50 µl** | **50 µl** | **50 µl** |

10) Incubate at 30°C for 45 minutes.

11) Thaw Kinase-Glo Max reagent.

12) During the incubation, thaw the Kinase-Glo Max™ reagent. At the end of the 45-minute reaction, add 50 µl of Kinase-Glo Max™ reagent to each well. Cover the plate with aluminum foil and incubate at room temperature for 15 minutes.

13) Measure luminescence using the microplate reader. Value of “Blank” reading should be subtracted from all other measurements.

**Example of Assay Results:**

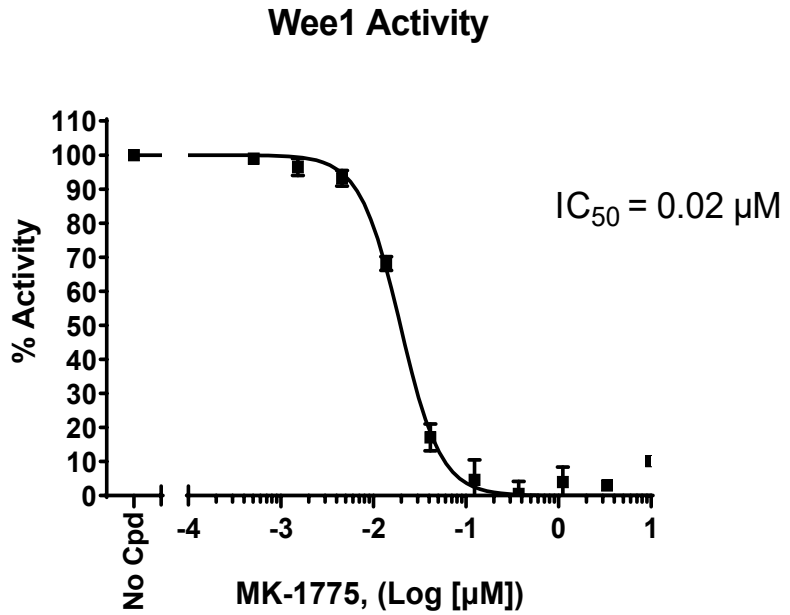


*Figure 1. Inhibition of Wee1 by MK-1775, measured using the Wee1 assay kit (BPS Bioscience #79909).*

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

**References**

1. Parker L, *et al*. *(1992) Science,* **257(5078)**: 1955.
2. Lundgreen K, *et al.* (1991) *Cell*, **64(6)**: 1111-1122.

**Related Products**

| *Products* | *Catalog #* | *Size* |
|---|---|---|
| Wee1, GST-Th-Tag Recombinant | 40412 | 10 µg |
| CDK1 Assay Kit | 79597 | 96 reactions |
| CRISPR/Cas9 Kinase Knockout Library (Array Format), Lentivirus | 78487 | 200 µl x 649 |
| Kinase (Human) CRISPR/Cas9 Lentivirus (Integrating) | 78488 | 200 µl x 2 |