Aurora Biolabs
High Purity Protein for Research

WEE1 Binding Assay Kit

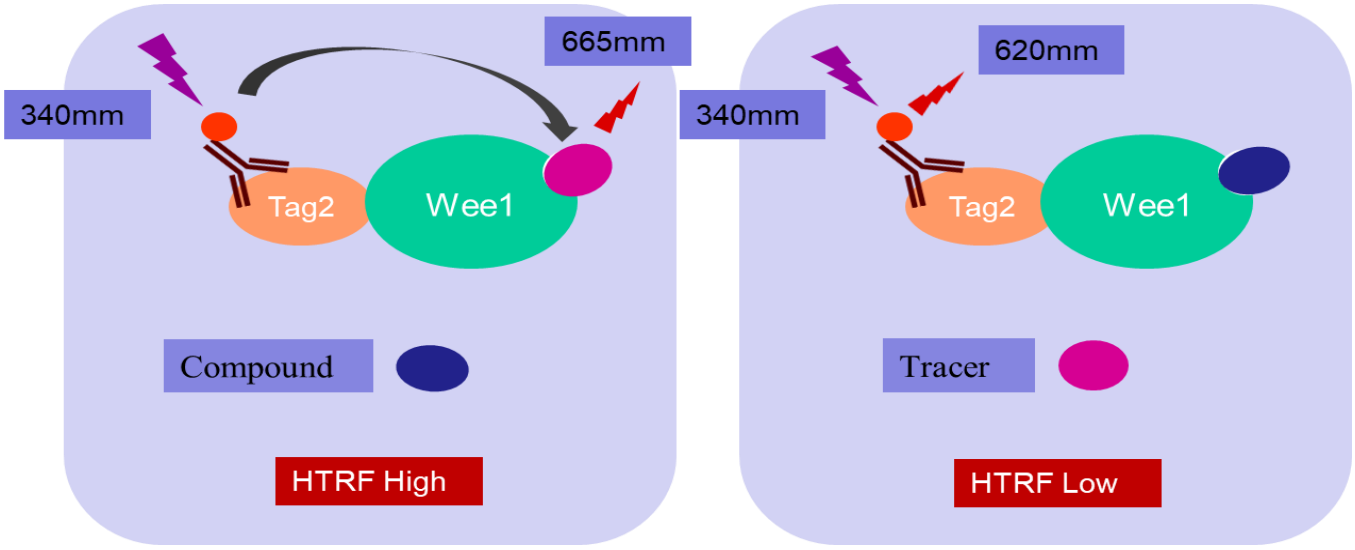
Catalog Number: 759331

Background

WEE1, a nuclear kinase, belongs to WEE kinase family that negatively regulates the cell cycle via phosphorylation of CDK1. WEE1 serves as a dual-specificity kinase which selectively phosphorylates both Thr14 and Tyr15 residues of both CDK1 and CDK2 to restrain their activation and halt cell cycle progression in the response to DNA damage. Overexpression of WEE1 is commonly observed in malignant cells and its high expression has been associated with poor rates of survival in various cancer types. Inhibition of WEE1 facilitates or even expedites mitotic progression, leading to an increase in genomic instability. Therefore, WEE1 is considered a potential therapeutic target for cancer treatment.

Assay Principle

The WEE1 binding assay kit is a TR-FRET based assay, which is designed to screen compounds that bind to WEE1. If the WEE1 with N-terminal tag2 binds to a fluorescence-labelled tracer (fluorescent receptor, emission at 665 mm), it brings the Terbium (fluorescence donor, emission at 620 mm) conjugated with anti-Tag2 antibody close to the fluorescent acceptor. Activation of the Terbium results in fluorescence resonance energy transfer (FRET), and leads to the receptor fluorescent emission at 665 mm. The competitive binding of a non-fluorescence-labeled compound will reduce the receptor signal. Thus, the compound binding status can be quantitively measured by calculating the ratio of the emission fluorescence intensity of the acceptor (665 nm) and donor (620 nm).



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Reference

Matheson, J.C., et al., Trends Pharmacol Sci. 2016 Oct;37(10):872-881.

Application

High throughput screening of compounds that inhibit WEE1 activity for drug discovery.

Plate Reader

A HTRF® certified microplate reader capable of measuring Time Resolved Fluorescence Resonance Energy Transfer (TR-FRET) is required.

Components

Catalog number	Item	Amount	Storage
759331-B	Assay buffer	20 mL	-20°C
759331-G	Full-length Human WEE1, Tag2	30 µL	-80°C
37882	Terbium-labeled anti-Tag2 antibody	20 µL	-80°C
37733	fluorescence-labeled tracer	80 µL	-80°C
	384-well microplate, White	1	Room temperature

Materials needed but not supplied

1. Microplate reader, HTRF® certified microplate reader
2. 0.5 M DTT
3. Adjustable micro-pipettor
4. Sterile Tips

Assay protocol

1. Prepare assay buffer containing 1 mM DTT

   For example, mix 998 µl of assay Buffer and 2 µl of 0.5 M DTT. Make only enough DTT-containing assay buffer as needed for the assay. Store the remaining assay buffer at -20°C.

2. Prepare the inhibitor compound solution

   If the inhibitor compound is dissolved in water, make a solution of the compound 10-fold higher than the final concentration in 1X assay buffer (since you will add 2 µl to the 20 µl reaction).

   If the inhibitor compound is dissolved in DMSO, make a 100-fold higher concentration of the compound than the highest concentration you want to test in DMSO. Then make a 10-fold dilution in 1X assay buffer (at this step, the compound concentration is 10-fold higher than the final concentration and the DMSO concentration is 10%). To determine an IC50 or to test lower concentrations of the compound, prepare as series of further dilutions in 1X assay buffer containing 10% DMSO (the final concentration of the DMSO will be 1% in all samples).

3. Prepare WEE1 solution

   Thaw WEE1 protein on ice. Upon first thaw, briefly spin the tube to recover all of the contents at the bottom of the tube. Make aliquots of the enzyme for single use. Store remaining undiluted protein at -80°C.

   Note: WEE1 protein is sensitive to freeze/thaw cycles. Limit number freeze-thaw cycles for best results. Do not store and re-use the diluted protein.

   Dilute the WEE1 protein 120-fold (1 µL WEE1 + 119 µL 1X assay buffer containing DTT).

   Add 8 µl of diluted protein solution to each positive control well and inhibitor test well.

   Add 8 µl of 1X DTT containing buffer to each of negative control well.

4. Add inhibitor

   Add 2 µl of diluted compound solution to each inhibitor test well.

   Add 2 µl of inhibitor solvent solution to each of negative and positive control well.

   Incubate at room temperature for 30 minutes (optional).

5. Prepare fluorescence-labeled tracer and Tb-labeled anti-Tag2 antibody solution

   Thaw the tracer and the antibody to room temperature.

   Dilute the tracer 50-fold and the antibody 200-fold with 1X assay buffer containing DTT. For example, add 4 µl of the tracer and 1 µl of the anti-tag2 antibody to 200 µl of 1X DTT containing assay buffer.

Add 10 µl of diluted tracer and antibody solution too each well.

Dilute just enough the tracer and the antibody for the use. Store the remaining undiluted tracer and the antibody at -80°C. Do not re-use the diluted tracer and antibody solution.

6. Incubate the reaction at room temperature for 60 minutes.
7. Measure fluorescent intensity

   HTRF compatible microplate reader is needed to measure fluorescent intensity of the samples. Fluorescent intensity should be measured twice:

   1. Excitation wavelength at 340 nm and emission at 620 nm.
   2. Excitation wavelength at 340 nm and emission at 665 nm.

Protocol Summary

Component	Negative Control	Positive Control	Inhibitor Test
1X buffer	8 µl		
WEE1		8 µl	8 µl
Inhibitor solvent	2 µl	2 µl	
Inhibitor solution			2 µl
Subtotal Volume	**10 µl**	**10 µl**	**10 µl**
Incubate at room temperature for 30 minutes (Optional).			
Fluorescence-labeled tracer and anti-Tag2 antibody solution	10 µl	10 µl	10 µl
Total Volume	**20 µl**	**20 µl**	**20 µl**
Incubate at room temperature for 60 minutes.			

Data Analysis

1. Calculate the ratio of the fluorescent intensity of each well.

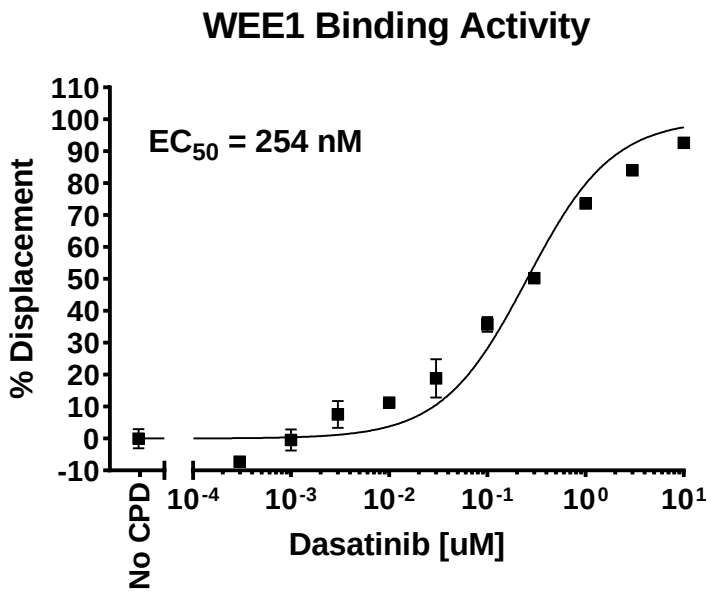
$$Ratio = \frac{\text{Fluorescent intensity at } 665 \text{ nm}}{\text{Fluorescent intensity at } 620 \text{ nm}}$$

2. Calculate percentage activity

   In the absence of the compound (positive control), the sample signal (P) is defined as 100% activity. In the absence of enzyme (negative control), the sample signal (N) is defined as 0% activity. The percent activity in the presence of each compound is calculated according to the following equation: % activity = (S-N)/(P-N) X100, where S= the sample signal in the presence of the compound.

$$\% \text{ Displacement} = (1 - (\frac{S - N}{\text{P} - \text{N}}))X100$$

Assay result



Related products:

Product Name	Catalog #	Size
DNA Polymerase Theta Activity Assay Kit	362101	96 reactions
DNA Polymerase Theta-N-Helicase Domain	7657643	20 ug, 100 ug
DNA Polymerase Theta-C terminal Domain	7657283	20 ug, 100 ug
DNA Polymerase Theta Full Length protein	7657385	10 ug, 50 ug
eIF4E/eIF4G Binding Assay Kit	34343-BK	384 reactions
PKMYT1 Binding Assay Kit	756981BK	384 reactions
WEE1 Binding Assay Kit	759331BK	384 reactions
T7 RNA polymerase	777627	5000 U, 25000U, 100000U
T7 High Yield RNA Synthesis Kit	777627-RK	25 rxns, 50 rxns, 100 rxns
Kras Wild Type (WT), GST-tag	5727-4121G	50 µg, 100 µg

Kras WT, GST-tag, GDP Loaded	5727-WTG-G	50 µg, 100 µg
Kras WT, GST-tag, GppNHp loaded	5727-WTG-GP	50 µg, 100 µg
Kras G12C, GST-tag	5727-4122G	50 µg, 100 µg
Kras G12C, GST-tag, GDP Loaded	5727-4122G -G	50 µg, 100 µg
Kras G12C, GST-tag, GppNHp loaded	5727-4122G -GP	50 µg, 100 µg
Kras G12D, GST-tag	5727-4123G	50 µg, 100 µg
Kras G12D, GST-tag, GDP Loaded	5727-4123G -G	50 µg, 100 µg
Kras G12D, GST-tag, GppNHp loaded	5727-4123G -GP	50 µg, 100 µg
Kras G12V, GST-tag,	5727-4128G	50 µg, 100 µg
Kras G12V, GST-tag, GDP Loaded	5727-4128G -G	50 µg, 100 µg
Kras G12V, GST-tag, GppNHp loaded	5727-4128G -GP	50 µg, 100 µg
Kras G13D, GST-tag,	5727-4133G	50 µg, 100 µg
Kras G13D, GST-tag, GDP Loaded	5727-4133G -G	50 µg, 100 µg
Kras G13D, GST-tag, GppNHp loaded	5727-4133G -GP	50 µg, 100 µg
Kras WT Nucleotide Exchange Assay Kit	5727-4121NK	384 reactions
Kras G12C Nucleotide Exchange Assay Kit	5727-4122NK	384 reactions
Kras G12D Nucleotide Exchange Assay Kit	5727-4123NK	384 reactions
Kras G12R Nucleotide Exchange Assay Kit	5727-4127NK	384 reactions
Kras G12V Nucleotide Exchange Assay Kit	5727-4128NK	384 reactions
Kras G13D Nucleotide Exchange Assay Kit	5727-4133NK	384 reactions
Kras WT – cRAF Binding Assay Kit	5727-4121BK	384 reactions
Kras G12C – cRAF Binding Assay Kit	5727-4122BK	384 reactions
Kras G12D– cRAF Binding Assay Kit	5727-4123BK	384 reactions
Kras G12R – cRAF Binding Assay Kit	5727-4127BK	384 reactions
Kras G12V – cRAF Binding Assay Kit	5727-4128BK	384 reactions
Kras G13D– cRAF Binding Assay Kit	5727-4133BK	384 reactions
Human RBD-RAF1, N-His tag, C-FLAG tag	7237231	50 µg, 100 µg
Human SOS1, No Tag	7671	50 µg, 100 µg
Human SOS1, His-Avi-Tag	7671HA	50 µg, 100 µg
Papain-like (PLpro) Protease Assay Kit	728253	96 reactions