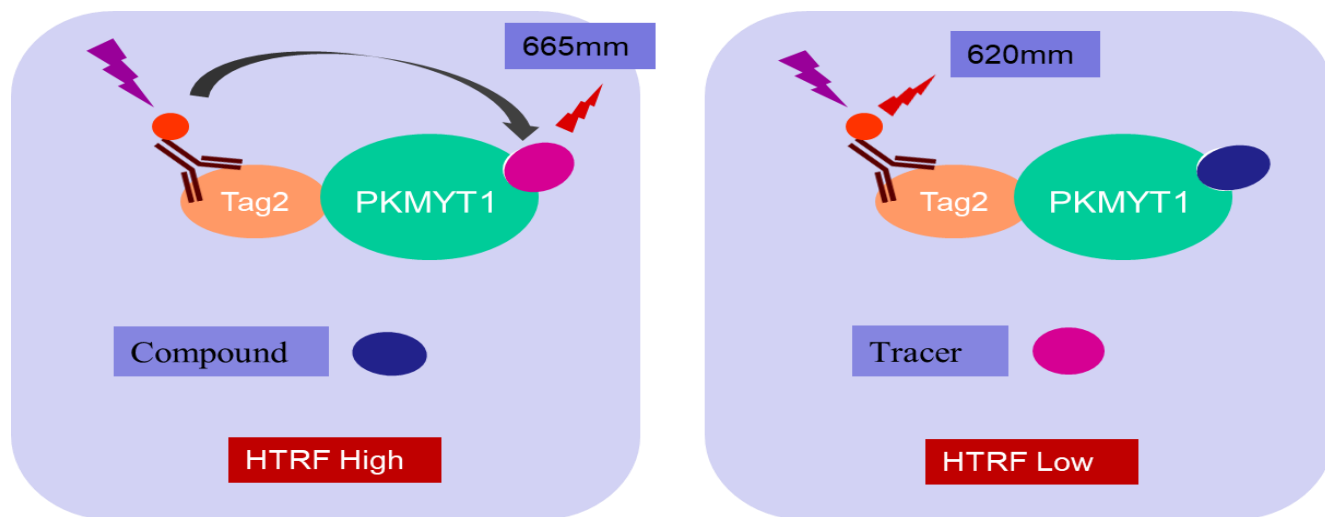


## Background

PKMYT1, a membrane-associated tyrosine- and threonine-specific cdc2-inhibitory kinase, belongs to WEE kinase family that plays an important role in the regulation of mitosis. PKMYT1 is involved in cell cycle progression and in response to DNA damages by inhibition of CDK1 activity through specific phosphorylation of Tyr15 and Thr14. Overexpression of PKMYT1 is observed in both solid and hematological tumors. Therefore, PKMYT1 is considered a potential therapeutic target for cancer treatment.

## Assay Principle

The PKMYT1 binding assay kit is a TR-FRET based assay, which is designed to screen compounds that bind to PKMYT1. A fluorescence-labelled tracer, that can bind to PKMYT1, and the N-terminal GST-tagged full-length human PKMYT1 are used in this assay kit. A Terbium-labeled anti-GST antibody binding to the GST-PKMYT1 serves as a fluorescence donor (HTRF donor). if the fluorescence-labeled tracer binds to the PKMYT1, the binding brings Terbium on the anti-GST antibody close to the fluorophore on the tracer (HTRF acceptor). Activation of the Terbium results in fluorescence resonance energy transfer (FRET). Thus, the binding status can be quantitatively measured by calculating the ratio of the emission fluorescence intensity of the acceptor (665 nm) and donor (620 nm). The competitive binding of a non-fluorescence-labeled compound will reduce the FRET signal.



## Application

High throughput screening of compounds that inhibit the PKMYT1 activity.

## 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          |
|----------------|------------------------------------|--------|------------------|
| 756981-B       | Assay buffer                       | 20 mL  | -20°C            |
| 756981-G       | Human PKMYT1, Tag2                 | 170 µL | -80°C            |
| 37882          | Terbium-labeled anti-Tag2 antibody | 20 µL  | -80°C            |
| 37733          | fluorescence-labeled tracer        | 40 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  $\mu$ l of assay Buffer and 2  $\mu$ l of 0.5 M DTT. Make only enough DTT-containing assay buffer as needed for the assay. Store the remaining assay buffer at  $-20^{\circ}\text{C}$ .

### 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  $\mu$ l to the 20  $\mu$ l 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  $\text{IC}_{50}$  or to test lower concentrations of the compound, prepare a 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 PKMYT1 solution

Thaw PKMYT1 protein on ice. Upon first thaw, briefly spin tube to recover the full contents at the bottom of the tube. Make aliquots of the enzyme for single use. Store remaining undiluted protein at  $-80^{\circ}\text{C}$ .

Note: PKMYT1 protein is sensitive to freeze/thaw cycles. Limit number freeze-thaw cycles for best results. Do not re-use the diluted protein.

Dilute the PKMYT1 protein 20-fold (1  $\mu$ L PKMYT1 + 19  $\mu$ L 1X assay buffer containing DTT).

Add 8  $\mu$ l of diluted protein solution to each positive control well and inhibitor test well.

Add 8  $\mu$ l of 1X DTT containing buffer to each of negative control well.

### 4. Add inhibitor

Add 2  $\mu$ l of diluted compound solution to each inhibitor test well.

Add 2  $\mu$ l 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 100-fold and the antibody 200-fold with 1X assay buffer containing DTT. For example, add 2  $\mu$ l of the tracer and 1  $\mu$ l of the anti-tag2 antibody to 200  $\mu$ l 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 the 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             |                  |                |
| PKMYT1   |                  | 8 µl             | 8 µl           |
| Inhibitor solvent  | 2 µl             | 2 µl             |                |
| Inhibitor solution   |                  |                  | 2 µl           |
| <b>Subtotal Volume</b>   | <b>10 µl</b>     | <b>10 µl</b>     | <b>10 µl</b>   |
| <b>Incubate at room temperature for 30 minutes (Optional).</b> |                  |                  |                |
| Fluorescence-labeled tracer and anti-Tag2 antibody solution    | 10 µl            | 10 µl            | 10 µl          |
| <b>Total Volume</b>  | <b>20 µl</b>     | <b>20 µl</b>     | <b>20 µl</b>   |
| <b>Incubate at room temperature for 60 minutes.</b>            |                  |                  |                |

## Data Analysis

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

$$Ratio1 = \frac{\text{Fluorescent intensity at 665 nm}}{\text{Fluorescent intensity at 620 nm}}$$

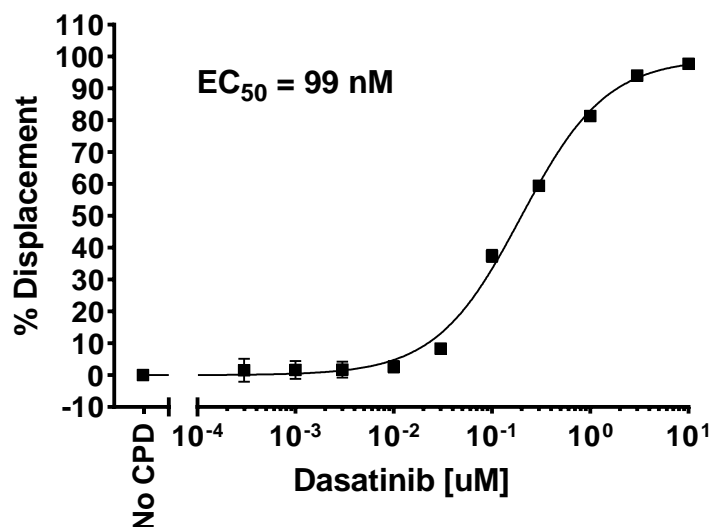
2. Calculate percentage activity

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.

$$\% \text{ Displacement} = \left(1 - \frac{S - N}{P - N}\right) \times 100$$

**Assay result**

**PKMYT1 Binding Activity**



**Related products:**

| <u>Product Name</u>                      | <u>Catalog #</u> | <u>Size</u>                |
|--|------------------|----------------------------|
| 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  |