

**Description**

The STK3 (MST2) Kinase Assay Kit is designed to measure STK3 (serine/threonine-protein kinase 3) kinase activity for screening and profiling applications using Kinase-Glo® MAX as a detection reagent. The assay kit comes in a convenient 96-well format, with enough purified recombinant STK3 (MST2) kinase, kinase substrate, ATP, and kinase assay buffer for 100 enzyme reactions.

**Background**

STK3, also known as serine/threonine-protein kinase 3 or MST2, is involved in the Hippo signaling pathway, a pathway crucial for cell development, proliferation and apoptosis and stress responses. STK3 is activated by autophosphorylation, which can be triggered by cellular stress signals. Once activated it can phosphorylate LATS1/LATS2 (large tumor suppressor kinase), which in turn inhibits YAP1 (yes-associated protein 1). It was found that kinases such as MAP4K (mitogen-activated protein kinase kinase kinase kinase) and TAOK (thousand and one kinase) function in parallel with STK3/4, depending on the cell type and stress inducer. Inhibited YAP1 cannot translocate to the cell nucleus and activate transcription, being targeted for degradation by the SCF (Skp, cullin, F-box containing) complex. Activated MST2 also phosphorylates FOXO (forkhead box O) factors and transcription of apoptotic genes. Interestingly, STK3 is inhibited by the proto-oncogene c-Raf, and this association is found in many cancers. STK3 can inhibit cancer progression, and it was found at low levels in gastric cancer and hepatocellular carcinoma. Conversely, high levels have also been found in breast cancer and oral squamous cell carcinoma. The development of inhibitors able to disrupt the interaction between c-Raf and STK3 may prove beneficial against some cancer types, but a better understanding of the opposite roles of STK3 in different cancers is still necessary.

**Applications**

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

**Supplied Materials**

Catalog #	Name	Amount	Storage
40013	STK3 (MST2), GST-Tag*	1 µg	-80°C
79334	5x Kinase Assay Buffer 1	1.5 ml	-20°C
79686	500 µM ATP	100 µl	-20°C
78514	Myelin Basic Protein (MBP) (5 mg/ml)	100 µl	-20°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® MAX DTT (Dithiothreitol), 1 M, optional Microplate reader capable of reading luminescence Adjustable micropipettor and sterile tips 30°C incubator	Promega #V6071

**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.
- The assay should include “Blank”, “Positive Control” and “Test Inhibitor” conditions.

1. Thaw **5x Kinase Assay Buffer 1**, **500 µM ATP** and **Myelin Basic Protein (MBP) (5 mg/ml)**.

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

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

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

3. Prepare a Master Mix (25 µl/well): N wells x (5 µl of 5x Kinase Assay Buffer 1 + 1 µl of 500 µM ATP + 1 µl of Myelin basic protein (MBP) (5 mg/ml) + 18 µl of distilled water).
4. Add 25 µl of Master Mix to every well.
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 **1x Kinase Assay Buffer 1**, 10-fold more concentrated than the desired final concentrations.

For the positive and negative controls, use **1x Kinase Assay Buffer 1** (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 1** 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 1 to keep the concentration of DMSO constant.

For positive and negative controls, prepare 10% DMSO in 1x Kinase Assay Buffer 1 (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  $\mu$ l of Test Inhibitor to each well labeled "Test Inhibitor".
7. Add 5  $\mu$ l of Diluent Solution to the "Positive Control" and "Blank" wells.
8. Add 20  $\mu$ l of 1x Kinase Assay Buffer 1 to the "Blank" wells.
9. Thaw **STK3 (MST2) kinase** on ice. Briefly spin the tube to recover its full content.
10. Dilute the protein kinase to 0.5 ng/ $\mu$ l with 1x Kinase Assay Buffer 1 (20  $\mu$ l/well).

*Note: The concentration of protein is lot-specific and is indicated on the tube. Verify the initial concentration and dilute accordingly. This kinase is particularly sensitive to freeze/thaw cycles. Avoid multiple freeze/thaw cycles. Do not re-use the thawed protein and do not re-use the diluted kinase.*

11. Initiate the reaction by adding 20  $\mu$ l of diluted kinase to the wells designated "Positive Control" and "Test Inhibitor".
12. Incubate at 30°C for 45 minutes.
13. Thaw the Kinase-Glo<sup>®</sup> Max reagent.
14. At the end of the 45-minute reaction, add 50  $\mu$ l of Kinase-Glo<sup>®</sup> Max reagent to each well.
15. Cover the plate with aluminum foil and incubate the plate at Room Temperature for 15 minutes.
16. Immediately read in a luminometer or a microplate reader capable of reading luminescence.

17. The “Blank” value should be subtracted from all other readings.

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 1	20 µl	-	-
Diluted STK3 (MST2) (0.5 ng/µl)	-	20 µl	20 µl
<b>Total</b>	<b>50 µl</b>	<b>50 µl</b>	<b>50 µl</b>

### Reading Luminescence

Luminescence is the emission of light resulting from a chemical reaction. The detection of luminescence requires no wavelength selection because the method used is emission photometry and not emission spectrophotometry.

To properly read luminescence, make sure the plate reader is set for LUMINESCENCE mode. Typical integration time is 1 second, delay after plate movement is 100 msec. Do not use a filter when measuring light emission. Typical settings for the Synergy 2 BioTek plate reader: use the “hole” position on the filter wheel; Optics position: Top; Read type: endpoint. Sensitivity may be adjusted based on the luminescence of a control assay without enzyme (typically we set this value as 100).

### Example Results

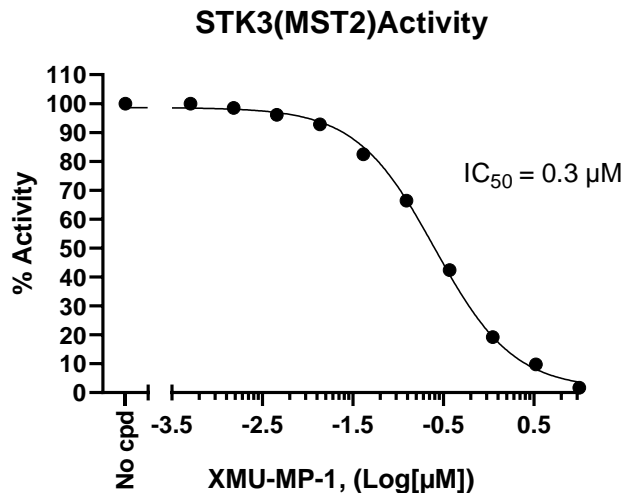


Figure 1: Inhibition of STK3 (MST2) kinase activity by XMU-MP-1.

STK3 (MST2) kinase activity was measured in the presence of increasing concentrations of XMU-MP-1 (Selleckchem #S8334). The Blank value was subtracted from all other values. Results are expressed as percent of control (kinase activity in the absence of inhibitor, set at 100%).

Data shown is representative. For lot-specific information, please contact BPS Bioscience, Inc. at [support@bpsbioscience.com](mailto:support@bpsbioscience.com).

**Troubleshooting Guide**

Visit [bpsbioscience.com/assay-kits-faq](https://bpsbioscience.com/assay-kits-faq) for detailed troubleshooting instructions. For all further questions, please email [support@bpsbioscience.com](mailto:support@bpsbioscience.com).

**References**

Yue L., *et al.*, 2023 *International Journal of General Medicine*, 16:3115-3124.

**Related Products**

<i>Products</i>	<i>Catalog #</i>	<i>Size</i>
STK33, GST-Tag Recombinant	40093	10 µg
LATS1, GST-Tag Recombinant	79499	10 µg
LATS2, GST-Tag Recombinant	79042	10 µg