

**Description**

The ITK Kinase Assay Kit is designed to measure ITK kinase activity for screening and profiling applications using ADP-Glo® as a detection reagent. The assay kit comes in a convenient 96-well format, with enough purified recombinant ITK kinase, kinase substrate, ATP and kinase assay buffer for 100 enzyme reactions.

**Background**

Tyrosine-protein kinase ITK/TSK, also known as Lck or IL2-inducible T cell kinase, is a cytosolic tyrosine kinase expressed in T cells. ITK contains protein binding domains SH2 and SH3, which allows the kinase to be recruited by tyrosine-phosphorylated proteins, notably upon activation of the T Cell Receptor (TCR). It plays an important role in TCR signal transduction pathways and ultimately leads to the activation of transcription factor NFAT (nuclear activator of activated T cells), cytokine secretion, and T cell differentiation and expansion.

**Applications**

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

**Supplied Materials**

Catalog #	Name	Amount	Storage
40445	ITK*	5 µg	-80°C
78426	Kinase assay buffer 2 (5x)	1.5 ml	-20°C
79686	ATP (500 µM)	100 µl	-20°C
40217	Poly-(Glu4:Tyr) (10 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	Catalog #
ADP-Glo® Kinase Assay	Promega #V6930
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

The **ADP-Glo™ Kinase Assay (Promega, #V6930)** quantifies the amount of ADP produced by a kinase upon phosphorylation of a substrate. First, addition of the ADP-Glo™ reagent terminates the reaction and quenches the remaining ATP. Second, addition of the Kinase Detection reagent converts the produced ADP to ATP. The new ATP is quantified by a luciferase reaction. The luminescent signal correlates with the amount of ADP generated by the kinase and is linear to 1 mM 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 2**, **ATP (500 μM)**, and **Poly-(Glu4:Tyr) (10 mg/ml)**.

*Optional: If desired, add DTT to **5x Kinase assay buffer 2** to make a 10 mM DTT concentration (Example: add 10 μl of 1 M DTT to 1 ml of **5x Kinase assay buffer 2**)*

2. Prepare 3 ml of **1x Kinase assay buffer** by mixing 600 μl of **5x Kinase assay buffer** with 2400 μl water.

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

3. Prepare the **Master Mix** (12.5 μl/well): N wells x (7 μl of **1x Kinase assay buffer 2** + 0.5 μl of **ATP (500 μM)** + 0.5 μl of Poly-(Glu4:Tyr) (10 mg/ml) + 4.5 μl of distilled water. Add 12.5 μl to every well.
4. Prepare the **Test Inhibitor** (2.5 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 25 μl.

If the Test Inhibitor is water-soluble:

- 4.1 Prepare serial dilutions in the **1x Kinase Assay Buffer 2**, 10-fold more concentrated than the desired final concentrations.
- 4.2 For the positive and negative controls, use **1x Kinase Assay Buffer 2** (Diluent Solution).

**Or**

If the Test inhibitor is soluble in DMSO:

- 4.1 Prepare the test inhibitor at 100-fold the highest desired concentration in DMSO, then dilute the inhibitor 10-fold in **1x Kinase Assay Buffer 2** to prepare the highest concentration of the 10-fold intermediate dilutions. The concentration of DMSO is now 10%.
- 4.2 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.

- 4.3 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%.*

5. Add 2.5  $\mu\text{l}$  of **Test Inhibitor** to each well labeled "Test Inhibitor." For the "Positive Control" and "Blank," add 2.5  $\mu\text{l}$  of **Diluent Solution** (either kinase assay buffer or 10% DMSO in kinase assay buffer, as described above).
6. To the wells designated as "Blank", add 10  $\mu\text{l}$  of **1x Kinase assay buffer**.
7. Thaw **ITK kinase** on ice. Briefly spin the tube to recover its full contents. Dilute the protein kinase (10  $\mu\text{l}$ /well) to 5 ng/ $\mu\text{l}$  using **1x Kinase assay buffer**.

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

*Note: 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.*

8. Initiate the reaction by adding 10  $\mu\text{l}$  of diluted Kinase to the wells designated "Positive Control" and "Test Inhibitor".

<b>Component</b>	<b>Blank</b>	<b>Positive Control</b>	<b>Test Inhibitor</b>
Master Mix	12.5 $\mu\text{l}$	12.5 $\mu\text{l}$	12.5 $\mu\text{l}$
Test Inhibitor	-	-	2.5 $\mu\text{l}$
Diluent Solution	2.5 $\mu\text{l}$	2.5 $\mu\text{l}$	-
1x Kinase Assay Buffer	10 $\mu\text{l}$		
ITK (5 ng/ $\mu\text{l}$ )	-	10 $\mu\text{l}$	10 $\mu\text{l}$
<b>Total</b>	<b>25 <math>\mu\text{l}</math></b>	<b>25 <math>\mu\text{l}</math></b>	<b>25 <math>\mu\text{l}</math></b>

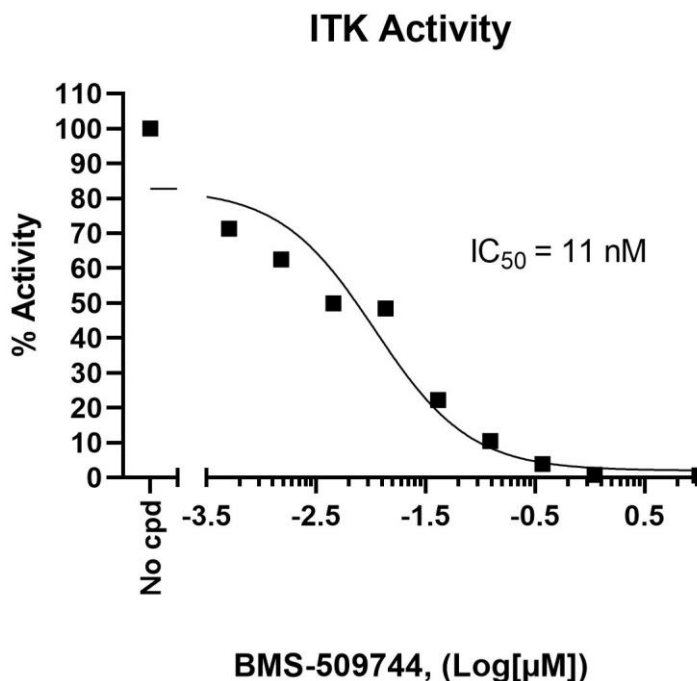
9. Incubate at 30°C for 45 minutes.
10. During the incubation, thaw the ADP-Glo™ reagent. At the end of the 45-minute reaction, add 25  $\mu\text{l}$  of ADP-Glo™ reagent to each well. Cover the plate with aluminum foil and incubate at room temperature for 45 minutes.
11. Thaw the Kinase Detection Reagent. At the end of the 45-minute incubation, add 50  $\mu\text{l}$  of Kinase Detection reagent to each well. Cover the plate with aluminum foil and incubate at room temperature for another 45 minutes.
12. Immediately read in a luminometer or a microplate reader capable of reading luminescence. The "Blank" value is subtracted from all other readings.

## 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 (typical value set =100).

## Example Results



*Figure 1: Inhibition of ITK kinase Activity by BMS-509744 (MedChemExpress #HY-11092).* The inhibition of ITK kinase activity was measured in the presence of increasing inhibitor concentrations using the ITK Kinase Assay Kit (BPS Bioscience #78429). 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%).

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

Weeks S, Harris R, Karimi M. Targeting ITK signaling for T cell-mediated diseases. *iScience*. 2021; **24(8)**: 102842.

**Related Products**

<i>Products</i>	<i>Catalog #</i>	<i>Size</i>
ITK, GST-tag Recombinant	40445	10 µg
Mouse ITK, GST-tag Recombinant	40446	10 µg
Rat ITK, GST-tag Recombinant	40447	10 µg
BTK Assay Kit	79568	96 reactions