

**Description**

The Chemi-Verse™ AKT1 Kinase Assay Kit is designed to measure AKT1 (protein kinase B) 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 AKT1 kinase, kinase substrate, ATP, and kinase assay buffer for 100 enzyme reactions.

**Background**

AKT1, also known as Protein Kinase B Alpha (PKB $\alpha$ ), is a serine/threonine kinase found in various cell types throughout the body, where it plays a critical role in regulating cell survival, insulin signaling, angiogenesis and tumor formation as well as controlling apoptosis. The signaling pathways involving AKT1 kinase are essential for the normal development and function of the nervous system. AKT1 has a broad number of targets and can thus regulate multiple pathways. Dysregulation of AKT1 contributes to a number of serious diseases, including cancer, diabetes, cardiovascular and neurological diseases. In cancer AKT1 is involved in tumor progression and drug resistance. For instance, the activating mutation E17K seems to be present in 7% of the estrogen-receptor positive metastatic breast cancers. The use of AKT inhibitors, such as capivasertib, alone or in combination, is currently under late-stage clinical trials and has been showing great promise for the treatment of cancer patients. A deep understanding of the mechanisms of action of AKT1 and how inhibition of AKT1 can lead to other drug resistance can be crucial to the development of new cancer therapies.

**Applications**

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

**Supplied Materials**

Catalog #	Name	Amount	Storage
40003	AKT1, Active*	2.5 $\mu$ g	-80°C
79334	5x Kinase Buffer 1	1.5 ml	-20°C
79686	500 $\mu$ M ATP	50 $\mu$ l	-20°C
	Crosstide (5 mg/ml)	50 $\mu$ l	-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
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, the addition of the Kinase Detection reagent converts the produced ADP to ATP. The newly generated 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.
- The assay should include “Blank”, “Positive Control” and “Test Inhibitor” conditions.
- We recommend maintaining the diluted protein on ice during use.
- For detailed information on protein handling please refer to [Protein FAQs \(bpsbioscience.com\)](http://bpsbioscience.com).
- We recommend using A-674563 as internal control. If not running a dose response curve for the control inhibitor, we recommend running the control inhibitor at 0.1X, 1X and 10X the IC<sub>50</sub> value shown in the validation data below.

1. Thaw **5x Kinase Assay Buffer 1**, **500 μM ATP**, and **Crosstide (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 2,400 μl of distilled water.

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

3. Prepare a **Master Mix** (12.5 μl/well): N wells x (6 μl of 5x Kinase Assay Buffer 1 + 0.5 μl of 500 μM ATP + 0.5 μl of Crosstide (5 mg/ml) + 5.5 μl of distilled water).
4. Add 12.5 μl of Master Mix to every well.
5. 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.

5.1 If the Test Inhibitor is water-soluble: Prepare serial dilutions in the **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 2.5 µl of Test Inhibitor to each well labeled "Test Inhibitor".
7. Add 2.5 µl of Diluent Solution to the "Positive Control" and "Blank" wells.
8. Add 10 µl of **1x Kinase Assay Buffer 1** to the "Blank" wells.
9. Thaw **AKT1 Kinase** on ice. Briefly spin the tube to recover its full content.
10. Dilute the protein kinase (10 µl/well) to 2.5 ng/µl in **1x Kinase Assay Buffer 1**.
11. Initiate the reaction by adding 10 µl of diluted kinase to the wells designated "Positive Control" and "Test Inhibitor".

Component	Blank	Positive Control	Test Inhibitor
Master Mix	12.5 µl	12.5 µl	12.5 µl
Test Inhibitor	-	-	2.5 µl
Diluent Solution	2.5 µl	2.5 µl	-
1x Kinase Assay Buffer 1	10 µl	-	-
Diluted AKT1 (2.5 ng/µl)	-	10 µl	10 µl
<b>Total</b>	<b>25 µl</b>	<b>25 µl</b>	<b>25 µl</b>

12. Incubate at 30°C for 45 minutes.
13. Thaw the ADP-Glo™ reagent.
14. At the end of the 45 minute reaction, add 25 µl of ADP-Glo™ reagent to each well.

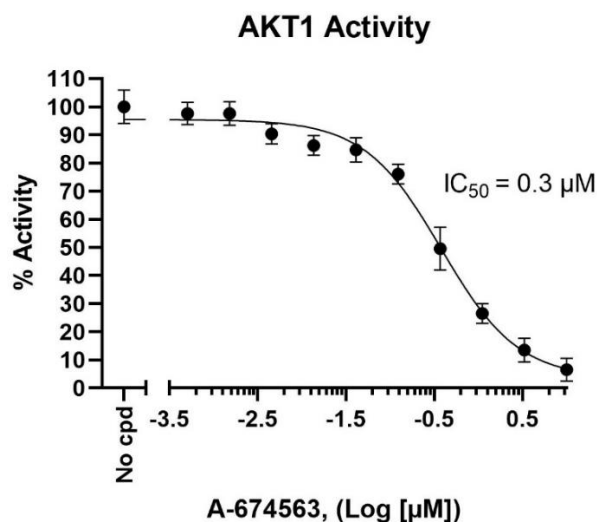
15. Cover the plate with aluminum foil and incubate at Room Temperature (RT) for 45 minutes.
16. Thaw the Kinase Detection Reagent.
17. Add 50  $\mu$ l of Kinase Detection reagent to each well.
18. Cover the plate with aluminum foil and incubate at RT for another 45 minutes.
19. Immediately read in a luminometer or a microplate reader capable of reading luminescence.
20. The “Blank” value should be 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 (typically we set this value as 100).

### Example Results



*Figure 1: Inhibition of AKT1 kinase activity by A-674563.*

AKT1 kinase activity was measured in the presence of increasing concentrations of A-674563 (SelleckChem #S1421). 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%).

*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**

Smyth L., *et al.*, 2020 *Clin Cancer Res* 26(15):3947-3957.

Hua H., *et al.*, 2021 *J Hematol Oncol* 14:128.

**Related Products**

<i>Products</i>	<i>Catalog #</i>	<i>Size</i>
AKT1, Inactive, His-Tag Recombinant	40000	25 µg/100 µg
AKT2, Inactive, His-Tag Recombinant	40001	100 µg
AKT1, Active, His-Tag Recombinant	40011	10 µg

*Version 120423*