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Description

The Chemi-Verse™ AMPK (A1/B1/G1) Kinase Assay Kit is designed to measure AMPK ((AMP-activated protein kinase) (A1/B1/G1)) 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 AMPK (A1/B1/G1) kinase, kinase substrate, ATP, and kinase assay buffer for 100 enzyme reactions.

Background

AMPK, or 5' AMP-activated protein kinase, is a heterotrimeric protein complex of α , β and γ subunits, which have specific roles. AMPK is ubiquitously expressed, playing a crucial role in the liver, brain and skeletal muscle. It is involved in the regulation of cellular energy by sensing the ratio AMP(ADP)/ATP in the cell. AMP binding to the γ subunit causes a conformational change, leading to the phosphorylation of threonine residues in the α subunits by AMPK kinase. This activation of AMPK leads to phosphorylation of proteins like ACC1 (acetyl-CoA carboxylase), SREBP1c (sterol regulatory element-binding protein 1c) or TBC1D1 (Rab-GTPase activating protein) and results in fatty acid oxidation, glucose uptake, lipogenesis, triglyceride and cholesterol synthesis and insulin secretion. Once the balance in the cells shifts from AMP to ATP, ATP dislodges AMP from AMPK and the protein becomes inactivated. The dysfunction of AMPK is involved in diabetes, obesity and NASH (nonalcoholic steatohepatitis). Its role in cancer, however, is controversial. It has been implicated in tumor suppression, as knockout models presented a tendency to develop lymphoma, but also in tumor promotion as in the absence of AMPK cancer cells became more prone to glucose starvation. Further studies are necessary for a full understanding of AMPK role in disease, and how to best modulate its activity.

Applications

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

Supplied Materials

Catalog #	Name	Amount	Storage
40025	AMPK (A1/B1/G1), His-Tag*	10 μ g	-80°C
79334	5x Kinase Buffer 1	1.5 ml	-20°C
79686	500 μ M ATP	50 μ l	-20°C
79496	5 mM AMP	50 μ l	-80°C
	AMARA peptide (1 mg/ml)	250 μ 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 new ATP generated 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.

1. Thaw **5x Kinase Assay Buffer 1, 500 μM ATP, 5 mM AMP** and **AMARA peptide (1 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 5 mM AMP + 2.5 μl of AMARA peptide (1 mg/ml) + 3 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 **AMPK (A1/B1/G1) kinase** on ice. Briefly spin the tube to recover its full content.
10. Dilute the protein kinase (10 µl/well) to 10 ng/µl in 1x Kinase Assay Buffer 1.

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 10 µl of diluted kinase to the wells designated "Positive Control" and "Test Inhibitor".
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 μ 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.

Component	Blank	Positive Control	Test Inhibitor
Master Mix	12.5 μ l	12.5 μ l	12.5 μ l
Test Inhibitor	-	-	2.5 μ l
Diluent Solution	2.5 μ l	2.5 μ l	-
1x Kinase Assay Buffer 1	10 μ l	-	-
Diluted AMPK (A1/B1/G1) (10 ng/ μ l)	-	10 μ l	10 μ l
Total	25 μl	25 μl	25 μl

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

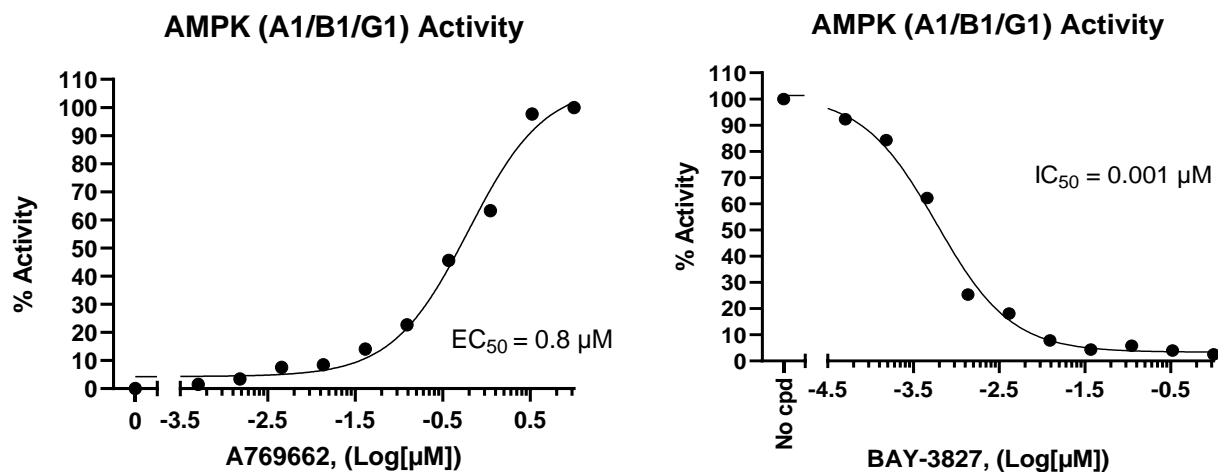


Figure 1: AMPK (A1/B1/G1) kinase activity in the presence of A769662 and BAY-3827. AMPK (A1/B1/G1) kinase activity was measured in the presence of increasing concentrations of the activator A769662 (Tocris #3336) (left) and the inhibitor BAY-3827 (SelleckChem S9833) (right). Results are expressed as the percent of control (kinase activity in the presence of activator or inhibitor, set at 100%).

Data shown is representative. For lot-specific information, please contact BPS Bioscience, Inc. at support@bpsbioscience.com.

Troubleshooting Guide

Visit bpsbioscience.com/assay-kits-faq for detailed troubleshooting instructions. For further questions, email support@bpsbioscience.com.

References

Steinberg G. and Hardie D., 2023 *Nature Reviews Molecular Cell Biology* 24: 255(273)

Related Products

Products	Catalog #	Size
AMPK (A2/B1/G1), His-Tag Recombinant	40704	5 μg
AMPK (A1/B2/G1), His-Tag Recombinant	40703	5 μg
AMPK (A1/B1/G2), His-Tag Recombinant	40021	10 μg