Acetyl-Coenzyme A Carboxylase 1 (ACC1) Assay Kit

#79315
96 reactions

Description

The Acetyl-Coenzyme A Carboxylase (ACC1) Assay Kit is designed to measure ACC1 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 ACC1, ATP, acetyl-CoA, sodium bicarbonate and assay buffer for 100 enzyme reactions.

Background

ACC1 (acetyl-coenzyme A carboxylase 1) is one of two isoforms of acetyl-CoA carboxylase. It is cytosolic and it is involved in ATP-dependent carboxylation of acetyl-CoA to malonyl-CoA, the rate-limiting step in *de novo* fatty acid synthesis, and it is found predominantly in the liver and adipose tissue. Its function is regulated by phosphorylation, allosteric regulators and other proteins, in response to the energetic needs of cells. Acetyl-CoA is at the crossroads between multiple metabolic pathways, so ACC1 has an impact in the formation of building blocks for new cells and in the response to metabolic stress. ACC1 has been linked to several diseases, such as cancer, diabetes, NAFLD (non-alcholic fatty liver disease) and obesity. Inhibition of ACC1 by TOFA (5-tetradecyloxy-2-furoic acid) can result in complete blockage of DNL (*de novo* lipogenesis) and may be a potential therapy for patients with NAFLD. The development of inhibitors specific for ACC1, for instance by targeting their catalytic domains or dimerization, may prove beneficial in the treatment of ACC1-related diseases.

Applications

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

Supplied Materials

Catalog #	Name	Amount	Storage
50202	ACC1, FLAG-His-Tags*	10 µg	-80°C
79283	5x ACC Assay Buffer	1 ml	-20°C
79686	500 µM ATP	100 µl	-20°C
	2 mM Acetyl-CoA	25 µl	-20°C
	1M Sodium Bicarbonate	75 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
ADP-Glo™ Kinase Assay	Promega #V6930
Microplate reader capable of reading luminescence	
Adjustable micropipettor and sterile tips	

Storage Conditions

This assay kit will perform optimally for up to **6 months** from date of receipt when the materials are stored as directed.

BPS Bioscience
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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.

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", "No Substrate Control", "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).
- We recommend using PF-05175157 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_{50} value shown in the validation data below.

1. Thaw **5x ACC Assay Buffer, 500 µM ATP, 1 M Sodium Bicarbonate** and **2 mM Acetyl-CoA.**

2. Prepare a **Master Mix** (15 µl/well, except "No Substrate Control" wells): N wells x (3.5 µl of 5x ACC Assay Buffer + 1 µl of 500 µM ATP + 0.25 µl of 2 mM Acetyl-CoA + 0.75 µl of 1M Sodium Bicarbonate + 9.5 µl of distilled water).

3. Add 15 µl of Master Mix to every well, except the "No Substrate Control" wells.

4. Prepare a **Deficient Master Mix** (15 µl/"No Substrate Control" wells): N wells x (3.5 µl of 5x ACC Assay Buffer + 1 µl of 500 µM ATP + 10.5 µl of distilled water).

5. Add 15 µl of Deficient Master Mix to the "No Substrate Control" wells.

6. Dilute 5x ACC Assay Buffer 5-fold with distilled water. This makes 1x ACC Assay Buffer.

   Note: 2 ml of 1x ACC Assay Buffer is enough for 100 reactions.

7. 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.

   7.1 If the Test Inhibitor is water-soluble: Prepare serial dilutions in distilled water, 10-fold more concentrated than the desired final concentrations.

   For the positive and negative controls, use distilled water (Diluent Solution).

OR

7.2 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 distilled water 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 distilled water to keep the concentration of DMSO constant.

For positive and negative controls, prepare 10% DMSO in distilled 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%.

8. Add 2.5 µl of Test Inhibitor to each well labeled “Test Inhibitor”.

9. Add 2.5 µl of Diluent Solution to the “Positive Control", “No Substrate Control” and “Blank” wells.

10. Add 7.5 µl of 1x ACC Assay Buffer to the "Blank" wells.

11. Thaw **ACC1** on ice. Briefly spin the tube to recover its full content.

12. Dilute ACC1 (7.5 µl/well) to 11 ng/µl with **1x ACC Assay Buffer**.

13. Initiate the reaction by adding 7.5 µl of diluted ACC1 to the wells designated “Positive Control”, “No Substrate Control” and "Test Inhibitor".

Component	Blank	No Substrate Control	Positive Control	Test Inhibitor
Master Mix	15 µl	-	15 µl	15 µl
Deficient Master Mix	-	15 µl	-	-
Test Inhibitor	-		-	5 µl
Diluent Solution	5 µl	5 µl	5 µl	-
1x ACC Assay Buffer	7.5 µl	-	-	-
Diluted ACC1 (11 ng/µl)	-	7.5 µl	7.5 µl	7.5 µl
Total	**25 µl**	**25 µl**	**25 µl**	**25 µl**

14. Incubate at Room Temperature (RT) for 40 minutes.

15. Thaw the ADP-Glo™ reagent.

16. At the end of the 40 minute reaction, add 25 µl of ADP-Glo™ reagent to each well.

17. Cover the plate with aluminum foil and incubate RT for 45 minutes.

18. Thaw Kinase Detection Reagent.

19. Add 50 µl of Kinase Detection Reagent to each well.

20. Cover the plate with aluminum foil and incubate RT for 45-90 minutes.

21. Immediately read in a luminometer or a microplate reader capable of reading luminescence.

22. 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 (typically we set this value as 100).

Example Results

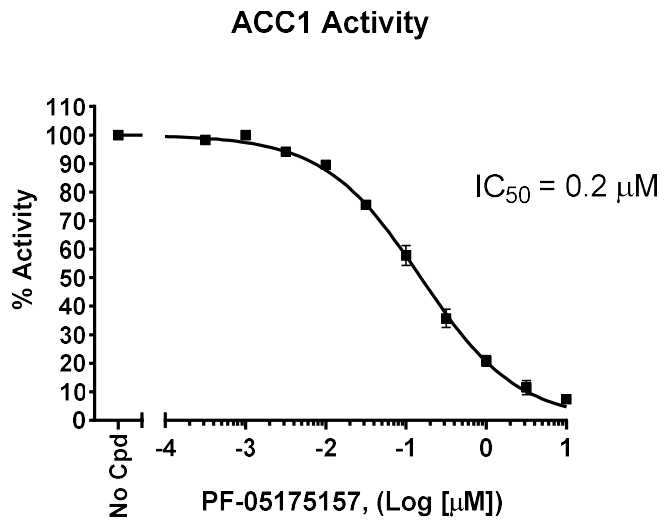

Figure 1: Inhibition of ACC1 activity by PF-05175157.
ACC1 activity was measured in the presence of increasing concentrations of PD-05175157. The "Blank" value was subtracted from all other values. Results are expressed as the percent of control (kinase activity in the absence of inhibitor, set at 100%).

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

Troubleshooting Guide

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

References

Tong L. J., 2006 *Cell. Biochem.* 99(6):1476-1488.
Li S., *et al.*, 2022 *Nature Communications* 13: 3998.
Wang Y., *et al.*, 2022 *Front Oncol.* 12: 836058.

Related Products

Products	*Catalog #*	*Size*
ACC1, His-Tag Recombinant	101991	10 µg/25 µg
ND-630	27071	1 mg/5 mg
Acetyl-Coenzyme A Carboxylase 2 (ACC2) Assay Kit	79282	96 reactions
ACC2, His-Tag Recombinant	50201	10 µg
Chemi-Verse™ CDK8/Cyclin C Kinase Assay Kit	78886	96 reactions

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