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Data Sheet

Acetyl-Coenzyme A Carboxylase 2 (ACC2) Assay Kit

Cat # 79282

DESCRIPTION: Acetyl-Coenzyme A Carboxylase (ACC) plays an important role in fatty acid metabolism so it has been proposed to be a drug target for the fatty acid-related metabolic diseases including obesity and diabetes. The *Acetyl-Coenzyme A Carboxylase 2 (ACC2) assay kit* is designed to measure ACC2 activity for screening and profiling applications using ADP-Glo™ reagents as a detection reagent. The *Acetyl-coenzyme A carboxylase 2 (ACC2) assay kit* comes in a convenient 96-well format, with enough purified recombinant ACC2 enzyme, ACC2 substrate, ATP and ACC assay buffer for 100 enzyme reactions.

COMPONENTS:

Catalog #	Reagent	Amount	Storage	
50101	ACC2	5 µg	-80°C	***Avoid multiple freeze/ thaw cycles!***
79283	5x ACC assay buffer	1 ml	-20°C	
	ATP (100 µM)	500 µl	-20°C	
	Acetyl-CoA (2 mM)	25 µl	-20°C	
	Sodium Bicarbonate (400 mM)	75 µl	-20°C.	
	96-well plate, white	1	Room Temp.	

MATERIALS OR INSTRUMENTS REQUIRED BUT NOT SUPPLIED:
ADP-Glo™ Kinase Assay (Promega #V6930)
Microplate reader capable of reading luminescence
Adjustable micropipettor and sterile tips

APPLICATIONS: Useful for studying enzyme kinetics and screening small molecular inhibitors for drug discovery and HTS applications.

STABILITY: Up to 6 months when stored as recommended.

REFERENCE:

Ting, L. *J. Cell. Biochem.* **99(6):**1476-1488 (2006)

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ASSAY PROTOCOL:
All samples and controls should be tested in duplicate.

1) Thaw 5x ACC assay buffer, ATP and Acetyl-CoA and Sodium Bicarbonate.

2) Prepare the master mixture (15 µl per well): N wells x (3.5 µl 5x ACC assay buffer + 5 µl ATP (100 µM) + 0.25 µl Acetyl-CoA + 0.75 µl Sodium Bicarbonate + 5.5 µl water). Add 15 µl to every well except the Blank well.

3) For the Blank well, prepare 'no substrate master' (15 µl per well): N wells x (3.5 µl 5x ACC assay buffer + 5 µl ATP (100 µM) +6.5 µl water). Add 15 µl to the Blank well. (Alternatively, '*master mix + no ACC2*' can be used as a Blank well)

	Positive Control	**Test Inhibitor**	**Blank** (no substrate)	**Blank** (no ACC2)
5x ACC assay buffer	3.5 µl	3.5 µl	3.5 µl	3.5 µl
ATP (100 µM)	5 µl	5 µl	5 µl	5 µl
Acetyl-CoA (2 mM)	0.25 µl	0.25 µl	–	0.25 µl
Sodium Bicarbonate (400 mM)	0.75 µl	0.75 µl	–	0.75 µl
Water	5.5 µl	5.5 µl	6.5 µl	5.5 µl
Test Inhibitor	–	2.5 µl	–	–
Inhibitor Diluent (no inhibitor)	2.5 µl	–	2.5 µl	2.5 µl
1x ACC buffer	–	–	–	7.5 µl
ACC2 (5.4 ng/µl)	7.5 µl	7.5 µl	7.5 µl	–
Total	25 µl	25 µl	25 µl	25 µl

4) Add 2.5 µl of Inhibitor solution of each well labeled as "Test Inhibitor". For the "Positive Control" and "Blank", add 2.5 µl of the same solution without inhibitor (Inhibitor Diluent; *e.g. 10% DMSO (aqueous) is recommended for inhibitor diluent, resulting in 1% DMSO at final concentration)*

5) Prepare 2 ml of 1x ACC assay buffer by mixing 400 µl of 5x ACC assay buffer with 1600 µl water. 2 ml of 1x Kinase assay buffer is sufficient for 100 reactions.

6) To the wells designated as "Blank (no ACC2)", add 7.5 µl of 1x ACC assay buffer.

7) Thaw ACC2 enzyme on ice. Upon first thaw, briefly spin tube containing enzyme to recover full content of the tube. Calculate the amount of ACC2 required for the

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assay and dilute enzyme to ~5.4 ng/µl with 1x ACC assay buffer. Store remaining undiluted enzyme in aliquots at -80°C. *Note: ACC2 enzyme is sensitive to freeze/thaw cycles. Avoid multiple freeze/thaw cycles. Do not re-use thawed aliquots or diluted enzyme.*

8) Initiate reaction by adding 7.5 µl of diluted ACC2 enzyme to the wells designated "Positive Control", "Test Inhibitor Control" and "Blank (no substrate)". Incubate at room temperature for 40 minutes.

9) Thaw ADP-Glo reagent (Promega).

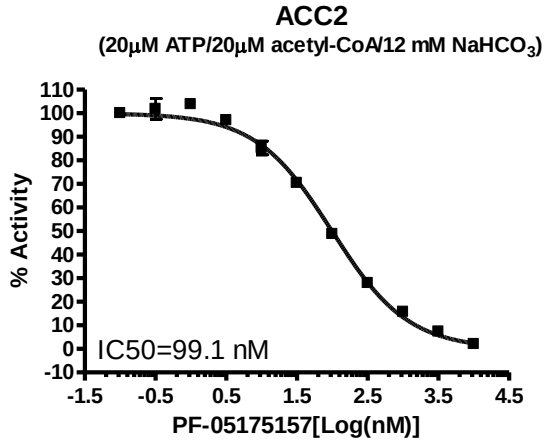
10) After the 40 minutes reaction, add 20 µl of ADP-Glo reagent to each well. Cover plate and incubate at room temperature for 45 minutes.

11) Thaw Kinase Detection reagent (Promega).

12) After 45 min incubation with ADP-Glo reagent, add 50 µl of Kinase-Detection reagent to each well. Cover plate with aluminum foil and incubate at room temperature for 45 ~ 90 minutes

13) Measure luminescence using the microplate reader.

Example of Assay Results:



Inhibition of ACC2 enzyme by PF-05175157, measured using the *Acetyl-Coenzyme A Carboxylase 2 (ACC2) assay kit* (Cat. #xxxxx). *Data shown is lot-specific. For lot-specific information, please contact BPS Bioscience, Inc. at info@bpsbioscience.com*

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RELATED PRODUCTS:

Product Name	Catalog #	Size
ACC2, His-tag	50201	10 µg
ACC2, His-tag, Strep-tag	50203	10 µg
ACC1, His-tag	50200	10 µg
ACC1, FLAG-His-tags	50202	10 µg