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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 (Human 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	
50201	ACC2 (Human)	5 µg	-80°C	Avoid multiple freeze/thaw cycles!
79283	5x ACC assay buffer	1 ml	-20°C	
79686	ATP (500 µM)	100 µl	-20°C	
	Acetyl-CoA (2 mM)	25 µl	-20°C	
	Sodium Bicarbonate (400 mM)	75 µl	-20°C.	
79696	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:

Tong, 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 + 1 µl ATP (500 µM) + 0.25 µl Acetyl-CoA + 0.75 µl Sodium Bicarbonate + 9.5 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 + 1 µl ATP (500 µM) + 10.5 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 (500 µM)	1 µl	1 µl	1 µl	1 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	9.5 µl	9.5 µl	10.5 µl	9.5 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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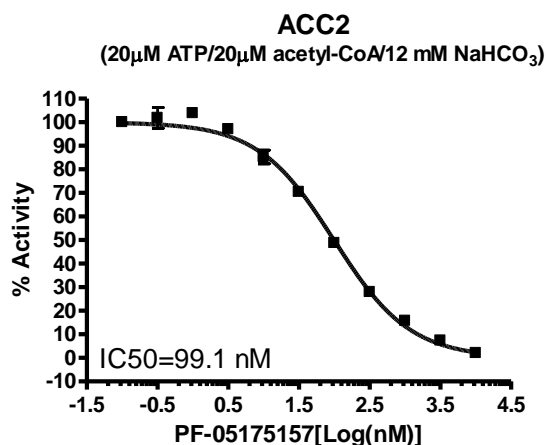
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assay and dilute enzyme to ~5.4 ng/μl with 1x ACC assay buffer. Store remaining undiluted enzyme in aliquots at -80°C. *Note: ACC2 enzyme is sensitive to freeze/thaw cycles. Avoid multiple freeze/thaw cycles. Do not re-use thawed aliquots or diluted 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 25 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:

<u>Product Name</u>	<u>Catalog #</u>	<u>Size</u>
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

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