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Data Sheet

ACLY Assay Kit

Catalog # 79904
Size: 96 reactions

Description: ATP citrate lyase (ACLY) is an important enzyme in fatty acid synthesis and cancer metabolism. The *ACLY Assay Kit* is designed to measure ACLY activity for screening and profiling applications using ADP-Glo® Kinase Assay as a detection reagent. The *ACLY Assay Kit* comes in a convenient 96-well format, with enough purified recombinant ACLY enzyme, substrate, ATP, and kinase assay buffer for 100 enzyme reactions.

COMPONENTS:

Catalog #	Reagent	Amount	Storage	
50255	ACLY	3 µg	-80°C	***Avoid multiple freeze/ thaw cycles!***
79334	5x Kinase assay buffer	1.5 ml	-20°C	
79686	ATP (500 µM)	100 µl	-20°C	
	Sodium Citrate (10 mM)	50 µl	-80°C	
	Coenzyme A (10 mM)	50 µl	-80°C	
79696	96-well plate, white	1	Room Temp.	

MATERIALS OR INSTRUMENTS REQUIRED BUT NOT SUPPLIED:
ADP-Glo® Kinase Assay (Promega #V6930)
Dithiothreitol (DTT, 1 M; optional)
Microplate reader capable of reading luminescence
Adjustable micropipettor and sterile tips
30°C incubator

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:
Zaidi, N., J. Swinnen, and K. Smans. 2012. “ATP-Citrate Lyase: A Key Player in Cancer Metabolism.” *Cancer Research* **72 (15):**3709-14.

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

1) Thaw **5x Kinase assay buffer**, **ATP (500 µM),** and **Sodium Citrate (10 mM)**, **Coenzyme A (10 mM)**.
(Optional: If desired, add DTT to **5x Kinase assay buffer** to make a 10 mM concentration; *e.g.* add 10 µl of 1 M DTT to 1 ml **5x Kinase assay buffer**).

2) Prepare the master mixture (15 µl per well): N wells x (3 µl **5x Kinase assay buffer** + 5 µl **ATP (500 µM)** + 0.5 µl **Coenzyme A (10 mM)** + 0.5 µl **Sodium Citrate (10 mM)** + 6 µl distilled water. Add 15 µl to every well.

	Positive Control	Test Inhibitor	Blank
5x Kinase assay buffer	3 µl	3 µl	3 µl
ATP (500 µM)	5 µl	5 µl	5 µl
Coenzyme A (10 mM)	0.5 µl	0.5 µl	0.5 µl
Sodium Citrate (10 mM)	0.5 µl	0.5 µl	0.5 µl
Distilled water	6 µl	6 µl	6 µl
Test Inhibitor	–	2.5 µl	–
10% DMSO in water (Inhibitor buffer)	2.5 µl	–	2.5 µl
1x Kinase buffer	–	–	7.5 µl
ACLY (4 ng/µl)	7.5 µl	7.5 µl	–
Total	25 µl	25 µl	25 µl

3) Prepare 100x stock solution of test inhibitor in DMSO*. Dilute 1:10 with water. 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 10% DMSO in water (Inhibitor buffer). *Note: Keep DMSO concentration of the Test Inhibitor at ≤10%, as final DMSO concentration in the reaction should be ≤1%.*
*If test inhibitor is water soluble, prepare 10x solution in water, and use water for the inhibitor buffer.

4) Prepare 3 ml of **1x Kinase assay buffer** by mixing 600 µl of 5x Kinase assay buffer with 2400 µl water. 3 ml of **1x Kinase assay buffer** is sufficient for 100 reactions.

5) To the wells designated as "Blank," add 10 µl of **1x Kinase assay buffer**.

6) Thaw **ACLY** enzyme on ice. Upon first thaw, briefly spin tube containing enzyme to recover full content of the tube. Calculate the amount of **ACLY** required for the assay and dilute enzyme to 4 ng/µl with **1x Kinase assay buffer**. Store

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remaining undiluted enzyme in aliquots at -80°C. *Note: ACLY enzyme is sensitive to freeze/thaw cycles. Avoid multiple freeze/thaw cycles. Do not re-use thawed aliquots or diluted enzyme.*

7) Initiate reaction by adding 7.5 µl of diluted **ACLY** enzyme to the wells designated "Positive Control" and "Test Inhibitor Control." Incubate at 30˚C for 45 minutes.

8) Thaw ADP-Glo reagent.

9) After the 45 minutes reaction, add 25 µl of ADP-Glo reagent to each well. Cover plate with aluminum foil and incubate the plate at room temperature for 45 minutes.

10) Thaw Kinase Detection reagent.

11) After the 45 minutes incubation, add 50 µl of Kinase Detection reagent to each well. Cover plate with aluminum foil and incubate the plate at room temperature for another 45 minutes.

12) Immediately read sample in a luminometer or microtiter-plate capable of reading chemiluminescence. "Blank" value is subtracted from all readings.

Reading Chemiluminescence:

Chemiluminescence is the emission of light (luminescence) which results from a chemical reaction. The detection of chemiluminescence requires no wavelength selection because the method used is emission photometry and is not emission spectrophotometry.

To properly read chemiluminescence, 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 are: 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).

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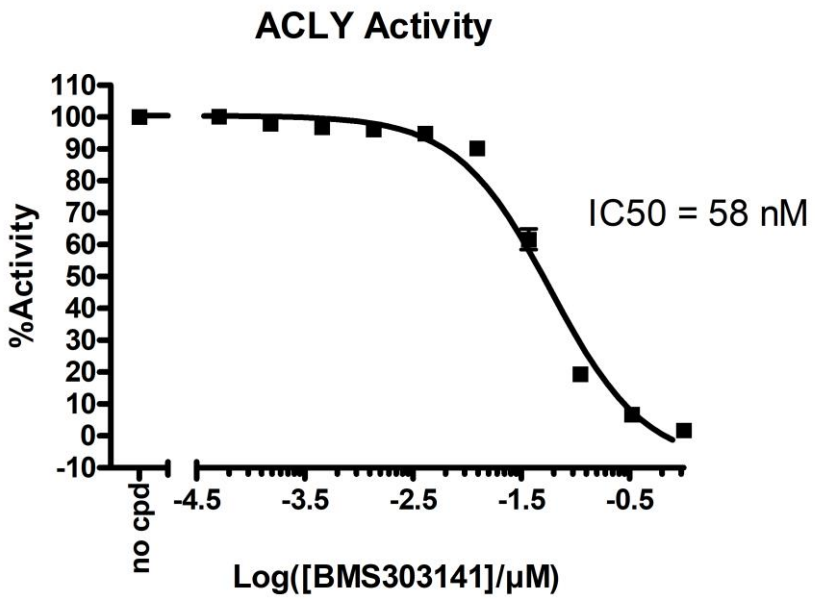
Example of Assay Results:



Inhibition of ACLY enzyme by BMS303141, measured using the ACLY Assay Kit, BPS Bioscience #79904. Data shown is lot-specific. For lot-specific information, please contact BPS Bioscience, Inc. at support@bpsbioscience.com

RELATED PRODUCTS:

Product Name	Catalog #	Size
ATP Citrate Lyase, GST-tag	50255	10 µg
ACC1, His-tag	50200	10 µg
ACC1, FLAG-tag	50202	10 µg
ACC2, His-tag	50201	10 µg
ATP (500 µM)	79686	200 µl