Amplite™ Colorimetric Oxaloacetate Assay Kit *Red Color*

Catalog number: 13840
Unit size: 200 Tests

Component	Storage	Amount
Component A: Amplite™ Red Substrate (light sensitive)	Freeze (< -15 °C), Minimize light exposure	1 vial
Component B1: Enzyme Mix 1	Freeze (< -15 °C), Minimize light exposure	2 bottles (lyophilized powder)
Component B2: Enzyme Mix 2	Freeze (< -15 °C), Minimize light exposure	2 vials (lyophilized powder)
Component C: Assay Buffer	Freeze (< -15 °C)	1 bottle (20 mL)
Component D: Oxaloacetate Decarboxylase (OAC)	Freeze (< -15 °C), Minimize light exposure	2 vials (lyophilized powder)
Component E: Oxaloacetate Standard	Freeze (< -15 °C), Minimize light exposure	1 vial (lyophilized powder)
Component F: DMSO	Freeze (< -15 °C)	1 vial (100 µL)

OVERVIEW

Oxaloacetate is an important part of citric acid cycle, where it reacts with Acetyl-CoA to form citrate. It is also involved in gluconeogenesis, urea cycle, glyoxylate cycle, amino acid synthesis, and fatty acid synthesis. The lack of oxaloacetate limits gluconeogenesis and urea cycle function, and can lead to decreased production of energy. Oxaloacetate can be also used as blood glutamate scavengers to provide neuroprotection after traumatic brain injury, expressed both by reduced neuronal loss in the hippocampus and improved neurologic outcomes. Amplite™ Colorimetric Oxaloacetate Assay Kit offers a sensitive assay for quantifying oxaloacetate in biological samples. Oxaloacetate is converted to pyruvate that generates hydrogen peroxide through an enzyme coupled reaction. The production of hydrogen peroxide is monitored with Amplite™ Red by an absorbance microplate reader at 575 nm.

AT A GLANCE

Protocol Summary

1. Prepare test samples and diluted oxaloacetate standards (50 µL)
2. Add equal volume of working solution (50 µL)
3. Incubate at RT for 30 minutes to 1 hour
4. Monitor absorbance intensity at 575 nm

Important Thaw all the kit components at room temperature before starting the experiment.

KEY PARAMETERS

Absorbance microplate reader

Absorbance	575 nm
Recommended plate	Clear bottom

PREPARATION OF STOCK SOLUTIONS

Unless otherwise noted, all unused stock solutions should be divided into single-use aliquots and stored at -20 °C after preparation. Avoid repeated freeze-thaw cycles.

1. Oxaloacetate standard solution (100 mM)
Add 100 µL of ddH_2O into Oxaloacetate Standard vial (Component E) to make 100 mM oxaloacetate standard solution.

2. Amplite™ Red substrate stock solution (200X)
Add 50 µL of DMSO (Component F) into Amplite™ Red substrate (Component A) to make 200X Amplite™ Red substrate stock solution.

PREPARATION OF STANDARD SOLUTION

For convenience, use the Serial Dilution Planner:
https://www.aatbio.com/tools/serial-dilution/13840

Oxaloacetate standard
Add 10 µL of 100 mM oxaloacetate into 990 µL of assay buffer (Component C) to get 1 mM oxaloacetate solution. Take 320 µL of 1 mM oxaloacetate standard solution into 680 µL assay buffer to make 320 µM oxaloacetate solution (OOA7). Then perform 1:2 serial dilutions to get remainder of serially diluted oxaloacetate standards (OOA6 - OOA1).

PREPARATION OF WORKING SOLUTION

1. Add 50 µL of ddH2O into Oxaloacetate Decarboxylase (Component D) to make 100X Oxaloacetate Decarboxylase solution.
2. Add 5 mL of Assay Buffer (Component C) into one Enzyme Mix 1 bottle (Component B1) and mix well.
3. Add 100 µL of ddH2O into one Enzyme Mix 2 vial (Component B2) and mix well.
4. Transfer 25 µL of 200X Amplite™ Red stock solution, 50 µL of OAC stock solution (from 1), and the entire vial (100 µL) of Enzyme Mix 2 (from 3) into the Enzyme Mix 1 bottle and mix well.

Note Avoid direct exposure to light and use promptly.

SAMPLE EXPERIMENTAL PROTOCOL

Table 1. Layout of oxaloacetate standards and test samples in a clear bottom 96-well microplate. OAA= Oxaloacetate Standard (OOA1 - OOA7, 5 to 320 µM), BL=Blank Control (assay buffer), TS=Test Sample.

BL	BL	TS	TS
OOA1	OOA1	...	...
OOA2	OOA2	...	...
OOA3	OOA3		
OOA4	OOA4		
OOA5	OOA5		
OOA6	OOA6		
OOA7	OOA7		

Table 2. Reagent composition for each well.

Well	Volume	Reagent
OOA1 - OOA7	50 µL	Serial Dilution (5 to 320 µM)
BL	50 µL	Assay Buffer (Component C)
TS	50 µL	Test Sample

1. Prepare oxaloacetate standards (OOA), blank controls (BL), and test samples (TS) according to the layout provided in Table 1 and Table 2. For a 384-well plate, use 25 µL of reagent per well instead of 50 µL.

2. Add 50 µL of assay working solution into each well of oxaloacetate standard, blank control, and test samples to make the total oxaloacetate assay volume of 100 µL/well. For a 384-well plate, add 25 µL of assay working solution into each well instead, for a total volume of 50 µL/well.

Note Run the oxaloacetate assay at pH 6.5 to 7.0.

3. Incubate the reaction at room temperature for 30 minutes to 1 hour.

4. Monitor the absorbance increase with an absorbance plate reader at 575 nm.

EXAMPLE DATA ANALYSIS AND FIGURES

The reading (Absorbance) obtained from the blank standard well is used as a negative control. Subtract this value from the other standards' readings to obtain the base-line corrected values. Then, plot the standards' readings to obtain a standard curve and equation. This equation can be used to calculate Oxaloacetate samples. We recommend using the Online Linear Regression Calculator which can be found at:

https://www.aatbio.com/tools/linear-logarithmic-semi-log-regression-online-calculator

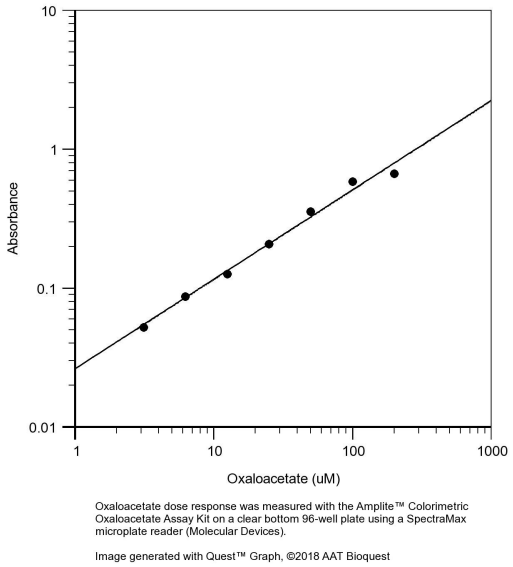

Figure 1. Oxaloacetate dose response was measured with the Amplite™ Colorimetric Oxaloacetate Assay Kit on a clear bottom 96-well plate using a SpectraMax microplate reader (Molecular Devices).

DISCLAIMER

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