Amplite™ Fluorimetric Oxaloacetate Assay Kit *Red Fluorescence*

Catalog number: 13841
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 Decarboxcylase (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™ Fluorimetric 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 a fluorescence microplate reader.

AT A GLANCE

Protocol summary

1. Prepare Oxaloacetate standards or test samples (50 µL)
2. Add Oxaloacetate working solution (50 µL)
3. Incubate at room temperature for 30 - 60 minutes
4. Monitor fluorescence increase at Ex/Em = 540/590 nm (Cutoff = 570 nm)

Important Thaw one vial of each kit component at room temperature before starting the experiment.

KEY PARAMETERS

Instrument:	Fluorescence microplate reader
Excitation:	540 nm
Emission:	590 nm
Cutoff:	570 nm
Recommended plate:	Solid black

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 ddH2O into Oxaloacetate Standard (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.

3. Oxaloacetate Decarboxcylase (OAC) stock solution (100X):
Add 50 µL of ddH2O into Oxaloacetate Decarboxcylase (Component D) to make 100X Oxaloacetate Decarboxcylase stock solution.

PREPARATION OF STANDARD SOLUTION

Oxaloacetate standard

For convenience, use the Serial Dilution Planner:
https://www.aatbio.com/tools/serial-dilution/13841

Add 10 µL of 100 mM Oxaloacetate standard solution into 990 µL of Assay Buffer (Component C) to get 1 mM Oxaloacetate standard solution. Take 1 mM Oxaloacetate standard solution and perform 1:10 in Assay Buffer (Component C) to make 100 µM Oxaloacetate standard solution (SD7). Take 100 µM Oxaloacetate standard solution (SD7) and perform 1:3 serial dilutions in Assay Buffer (Component C) to get serially diluted Oxaloacetate standards (SD6 - SD1).

PREPARATION OF WORKING SOLUTION

1. Add 5mL Assay Buffer (Component C) into one Enzyme Mix1 bottle (Component B1) and mix well.

2. Add 100 µL of ddH2O into one Enzyme Mix2 vial (Component B2) and mix well.

3. Transfer 25 µL of 200X Amplite™ Red stock solution, 50 µL of 100X OAC stock solution, and entire vial (100 µL) of Enzyme Mix2 into the Enzyme Mix1 bottle and mix well to make Oxaloacetate working solution.

Note This Oxaloacetate working solution is not stable, use it promptly, and avoid direct exposure to light.

SAMPLE EXPERIMENTAL PROTOCOL

Table 1. Layout of Oxaloacetate standards and test samples in a solid black 96-well microplate. SD=Oxaloacelate standard (SD1 - SD7, 0.1 to 100 µM), BL=Blank Control, TS=Test Samples.

BL	BL	TS	TS
SD1	SD1	...	...
SD2	SD2	...	...
SD3	SD3		
SD4	SD4		
SD5	SD5		
SD6	SD6		
SD7	SD7		

Tel: 408-733-1055 | Fax: 408-733-1304 | Email: support@aatbio.com

Table 2. Reagent composition for each well.

Well	Volume	Reagent
SD1 - SD7	50 µL	Serial Dilutions (0.1 to 100 µM)
BL	50 µL	Assay Buffer (Component C)
TS	50 µL	test sample

1. Prepare Oxaloacetate standards (SD), blank controls (BL), and test samples (TS) according to the layout provided in Tables 1 and 2. For a 384-well plate, use 25 µL of reagent per well instead of 50 µL.
2. Add 50 µL of Oxaloacetate working solution to 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 Oxaloacetate 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 - 60 minutes, protected from light.
4. Monitor the fluorescence increase with a fluorescence plate reader at Ex/Em = 540/590 nm (Cutoff = 570 nm).

EXAMPLE DATA ANALYSIS AND FIGURES

The reading (RFU) 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

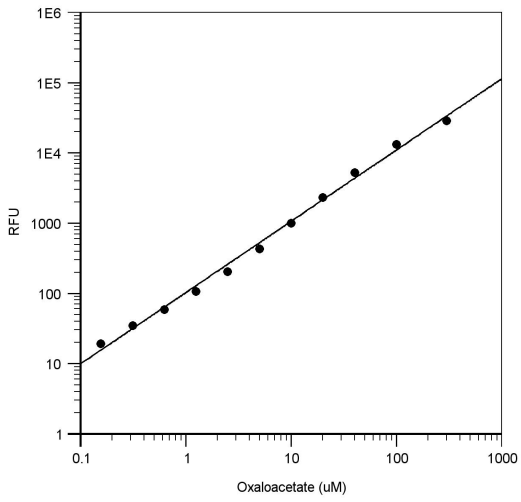

Figure 1. Oxaloacetate dose response was measured with the Amplite™ Fluorimetric Oxaloacetate Assay Kit on a solid black 96-well plate using a Gemini microplate reader (Molecular Devices).

DISCLAIMER