Amplite™ Fluorimetric Oxaloacetate Assay Kit

Red Fluorescence

Ordering Information:	*Storage Conditions:*	*Instrument Platform:*
Product Number: 13841 (200 assays)	Keep in freezer and protect from light	Fluorescence microplate readers

Introduction

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 Ex/Em = 540/590 nm.

Kit Components

Components	**Amount**
Component A: Amplite™ Red Substrate (light sensitive)	1 vial
Component B1: Enzyme Mix1	2 bottles (lyophilized powder)
Component B2: Enzyme Mix2	2 vials (lyophilized powder)
Component C: Assay Buffer	1 bottle (20 mL)
Component D: Oxaloacetate Decarboxylase (OAC)	2 vials (lyophilized powder)
Component E: Oxaloacetate Standard	1 vial (lyophilized powder)
Component F: DMSO	1 vial (100 µL)

Assay Protocol for One 96-well Plate

Brief Summary

Prepare test samples (50 µL) and serially diluted oxaloacetate standards (50 µL) → Add equal volume of Assay Mixture (50 µL) → Incubate at RT → Monitor fluorescence intensity at Ex/Em = 540/590 nm

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

1. Prepare serially diluted oxaloacetate standards and test samples:

1.1 Add 100 µL of dd H_2O into oxaloacetate standard (Component E) to make 100 mM oxaloacetate standard solution.

1.2 Prepare oxaloacetate standard dilutions: Add 10 µL of 100 mM oxaloacetate (from Step 1.1) into 990 µL of assay buffer (Component C) to get 1 mM oxaloacetate solution. Take 100 µL of 1 mM oxaloacetate standard solution into 900 µL assay buffer to make 100 µM oxaloacetate solution. Perform 1:3 serial dilutions to get approximately 30, 10, 3, 1, 0.3, and 0.1 µM serially diluted oxaloacetate standards.

1.3 Add 50 µL of oxaloacetate containing samples and serially diluted oxaloacetate standards into a solid black 96-well microplate according to Tables 1.

Table 1 Layout of oxaloacetate standards and test samples in a solid black 96-well microplate

BL	BL	TS	TS	….	….						
OAA1	OAA1	….	….	….	….						
OAA 2	OAA 2										
OAA 3	OAA 3										
OAA 4	OAA 4										
OAA 5	OAA 5										
OAA 6	OAA 6										
OAA 7	OAA 7										

Ordering: sales@aatbio.com; Tel: 800-990-8053 or 408-733-1055; Fax: 408-733-1304
Technical Support: support@aatbio.com; Tel: 408-733-1055

Note 1: OAA= Oxaloacetate Standard, BL=Blank Control (assay buffer), TS=Test Sample.
Note 2: Add the serial dilutions of oxaloacetate standard from 0.1 µM to 100 µM into wells from OAA1 to OAA7.

2. Prepare oxaloacetate Assay Mixture:

2.1 Make 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.

2.2 Make oxaloacetate decarboxcylase (OAC) stock solution (100X): Add 50 µL of dd H_2O into oxaloacetate decarboxcylase (Component D) to make 100 X oxaloacetate decarboxcylase stock solution.

2.3 Make Assay Mixture:

2.3.1 Add 5mL Assay Buffer (Component C) into one Enzyme Mix1 bottle (Component B1) and mix well.
2.3.2 Add 100 µL of ddH2O into one Enzyme Mix2 vial (Component B2) and mix well.
2.3.3 Transfer 25µL of 200X Amplite™ Red stock solution (from Step 2.1), 50 µL OAC stock solution (from step 2.2), and entire vial (100 µL) of Enzyme Mix2 (from Step 2.3.2) into the Enzyme Mix1 bottle (from Step 2.3.1) and mix well.
Note 1: The assay mixture is not stable, use it promptly, and avoid direct exposure to light.
Note 2: Store unused 200X Amplite™ Red stock solution at -20°C, avoid light and repeated freeze-thaw cycles.

3. Run oxaloacetate assay:

3.1 Add 50 µL of Assay Mixture (from Step 2.3) into each well of oxaloacetate standard, blank control, and test samples (see Step 1.3) to make the total oxaloacetate assay volume of 100 µL/well.
Note 1: For a 384-well plate, add 25 µL of sample, 25 µL of Assay mixture into each well.
Note 2: Run the oxaloacetate assay at pH 6.5 to 7.0.

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

3.3 Monitor the fluorescence increase with a fluorescence plate reader at Ex/Em=540/590 nm (cut off: 570 nm).

Data Analysis

The fluorescence reading in blank wells (with assay buffer only) is used as a control, and is subtracted from the values of those wells with the oxaloacetate standards and test samples. An oxaloacetate standard curve is shown in Figure 1. Calculate the oxaloacetate concentrations of the samples according to the oxaloacetate standard curve.

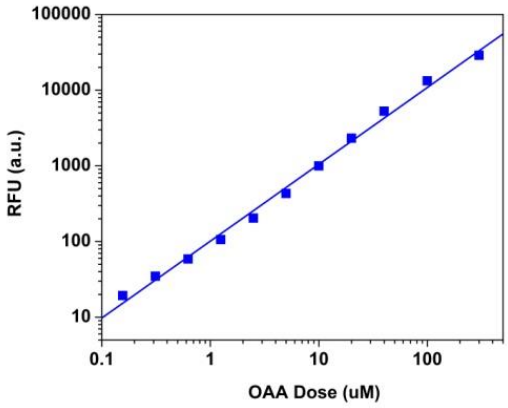

Figure1. Oxaloacetate dose response was measured with Amplite™ Fluorimetric Oxaloacetate Assay Kit (cat#13841) on a solid black 96-well plate using a Gemini microplate reader (Molecular Devices). As low as 0.3 µM oxaloacetate can be detected with 30min incubation (*Note: The fluorescence background increases with time, thus it is important to subtract the fluorescence intensity value of the blank wells for each data point).*

References

1. Iida, Katsumi. "Investigation of the biosynthesis of acetyl-CoA and oxaloacetic acid from pyruvic acid and the quantitative evaluation of incorporated 13C-labeled l-alanine in Arthrobacter hyalinus." *Journal of Radioanalytical and Nuclear Chemistry* (2014): 1-8.
2. Alexander Zlotnik, Igor Sinelnikov, Benjamin F. Gruenbaum, *et al.* "Effect of glutamate and blood glutamate scavengers oxaloacetate and pyruvate on neurological outcome and pathohistology of the hippocampus after traumatic brain injury in rats." Anesthesiology. 2012 January; 116(1): 73–83.
3. Mehta, Anuradha. "Studies on Oxalate decarboxylase from Collybia velutipes." (2014).