ab83428

Oxaloacetate Assay Kit

Instructions for Use

For the rapid, sensitive and accurate measurement of Oxaloacetate levels in various samples

This product is for research use only and is not intended for diagnostic use.

Version 3 Last Updated 4 February 2019

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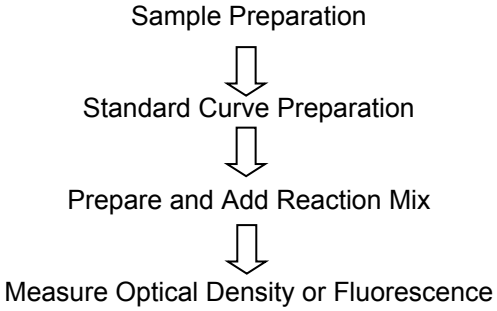
1. Overview

Oxaloacetate (OAA, HOOC-CO-CH_2-COOH) is a TCA cycle intermediate. It precedes citrate which is formed by the transfer of an acetyl group to OAA. OAA is formed by the deamidation of aspartate or condensation of CO_2 with pyruvate or PEP. Since mammals do not possess the enzymatic machinery to form TCA cycle intermediates from acetyl CoA, OAA is one of the anaplerotic entry points via pyruvate and pyruvate carboxykinase.

Abcam's Oxaloacetate Assay Kit provides a simple, sensitive and rapid means of quantifying OAA in a variety of samples. In the assay, OAA is converted to pyruvate which is utilized to convert a nearly colorless probe to an intensely colored (λ_{max}= 570nm) and fluorescent (Ex/Em = 535/587nm) product. The Oxaloacetate Assay Kit can detect 0.1-10nmol (2-200 µM) of OAA.

2. Protocol Summary



3. Components and Storage

A. Kit Components

Item	Quantity
OAA Assay Buffer	25 mL
OAA Probe	0.2 mL
OAA Enzyme Mix (Lyophilized)	1 vial
Developer (Lyophilized)	1 vial
OAA Standard (10 µmol, Lyophilized)	1 vial

* Store kit at -20°C, protect from light. Warm OAA Assay Buffer to room temperature before use.

OAA PROBE: Supplied ready to use (warm to >18°C to liquefy). Protect from light and moisture. Store at -20°C.

OAA ENZYME MIX, DEVELOPER: Add 220 µL OAA Assay Buffer to each component separately. Pipette up and down to dissolve. Aliquot into portions, store at -20°C. Avoid repeated freeze/thaw cycles. Use within 2 months.

OAA STANDARD: Add 100 µl dH_2O to generate 100 mM (100 nmol/µl) OAA Standard solution. Keep cold. Store at -20°C.

Note:

OAA slowly decomposes into pyruvate (several hours at room temp, several days at 0°C). This will not affect the assay.

B. Additional Materials Required

- Microcentrifuge
- Pipettes and pipette tips
- Fluorescent or colorimetric microplate reader
- 96 well plate
- Orbital shaker

4. Assay Protocol

1. Standard Curve Preparation:

a. For the colorimetric assay:

Dilute OAA Standard to1 nmol/µl by adding 10 µl of the standard to 990 µl of dH_2O, mix well. Add 0, 2, 4, 6, 8, 10 µl into a series of wells on a 96 well plate. Adjust volume to 50 µl/well with OAA Assay Buffer to generate 0, 2, 4, 6, 8, 10 nmol/well of the standard.

b. For the fluorometric assay:

Dilute OAA Standard to 0.1 nmol/µl by adding 10 µl of the standard to 990 µl of dH_2O, mix well, and then further dilute by adding 10 µl to 90 µl of dH_2O. Add 0, 2, 4, 6, 8, 10 µl into a series of standards well on a 96-well plate. Adjust volume to 50 µl/well to generate 0, 0.2, 0.4, 0.6, 0.8, 1.0 nmol/well OAA Standard.

2. Sample Preparation:

Tissue (20 mg) or cells (2 x 10^6) should be rapidly homogenized with 100µl OAA Assay Buffer. Centrifuge 15000 x g for 10 min to remove insoluble materials.

Note: Enzymes in samples may interfere with the assay. We suggest deproteinizing samples using 10 kDa molecular weight cut off spin columns (**ab93349**) or using a perchloric acid/KOH protocol as follows:

a) Tissue samples (20-1000 mg) should be frozen rapidly (liquid N_2 or methanol/dry ice), weighed and pulverized.

b) Add 2 µl 1N perchloric acid/mg per sample. KEEP COLD!

c) Homogenize or sonicate thoroughly. Spin homogenate at 10,000 x g for 5-10 minutes.

d) Neutralize supernatant with 3M $KHCO_3$, adding repeated 1 µl aliquots/10 µl supernate while vortexing. Add until bubble evolution ceases (2-5 aliquots). Put on ice for 5 minutes.

e) Check pH (using 1 µl) is ~6-8. Spin 2 minutes at 10,000 x g to pellet $KClO_4$.

Add 1-50 µl samples into duplicate wells of a 96-well plate and bring volume to 50 µl with Assay Buffer.

We suggest testing several doses of your samples to ensure readings are within the standard curve range.

3. Reaction Mix: Mix enough reagent for the number of samples and standards to be performed. For each well, prepare a total 50 µl Reaction Mix containing:

	Colorimetric Assay		**Fluorometric Assay**	
	Sample	**Sample Control***	**Sample**	**Sample Control***
Assay Buffer	44 µl	46 µl	44 µl	46 µl
Enzyme Mix	2 µl	---	2 µl	---
Developer	2 µl	2 µl	2 µl	2 µl
OAA Probe**	2 µl	2 µl	2 µl	2 µl

4. Add 50 µl of the Reaction Mix to each well containing the OAA Standard and test samples. Incubate for 30 minutes at room temperature, protect from light.

*** Note:** Pyruvate in samples can cause background color or fluorescence. This background can be subtracted by performing a sample control in the absence of the OAA Enzyme Mix.

**** Note:** In the fluorometric assay, dilute an aliquot of OAA Probe 10X with DMSO to reduce fluorescent background.

5. Measure OD at 570 nm or fluorescence Ex/Em at 535/587nm with a 96 well plate reader.

5. Data Analysis

Correct background by subtracting the value of the zero OAA standard from all readings. Next subtract the value of the Sample Control from the samples. The background reading can be significant and must be subtracted from sample readings.

Plot the standard curve. Apply corrected sample readings to the standard curve to get OAA amount in the sample wells.

The OAA concentrations in the test samples:

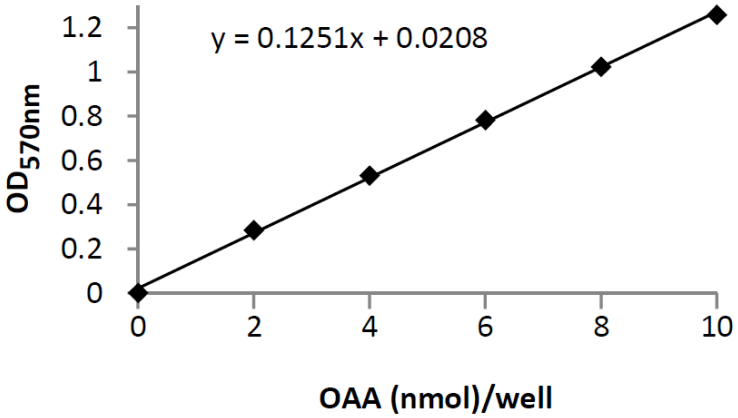
Concentration = Ay / Sv (nmol/µl; or µmol/ml; or mM)

Where:

Ay is the amount of OAA (nmol) in your sample from the standard curve.

Sv is the sample volume (µl) added to the sample well.

Oxaloacetic Acid molecular weight: 132.07 g/mol



Oxaloacetate standard curve generated following the kit protocol.

6. Troubleshooting

Problem	Reason	Solution
Assay not working	Assay buffer at wrong temperature	Assay buffer must not be chilled - needs to be at RT
	Protocol step missed	Re-read and follow the protocol exactly
	Plate read at incorrect wavelength	Ensure you are using appropriate reader and filter settings (refer to datasheet)
	Unsuitable microtiter plate for assay	Fluorescence: Black plates (clear bottoms); Luminescence: White plates; Colorimetry: Clear plates. If critical, datasheet will indicate whether to use flat- or U-shaped wells
Unexpected results	Measured at wrong wavelength	Use appropriate reader and filter settings described in datasheet
	Samples contain impeding substances	Troubleshoot and also consider deproteinizing samples
	Unsuitable sample type	Use recommended samples types as listed on the datasheet
	Sample readings are outside linear range	Concentrate/ dilute samples to be in linear range

Samples with inconsistent readings	Unsuitable sample type	Refer to datasheet for details about incompatible samples
	Samples prepared in the wrong buffer	Use the assay buffer provided (or refer to datasheet for instructions)
	Samples not deproteinized (if indicated on datasheet)	Use the ***10kDa spin column (ab93349)***
	Cell/ tissue samples not sufficiently homogenized	Increase sonication time/ number of strokes with the Dounce homogenizer
	Too many freeze-thaw cycles	Aliquot samples to reduce the number of freeze-thaw cycles
	Samples contain impeding substances	Troubleshoot and also consider deproteinizing samples
	Samples are too old or incorrectly stored	Use freshly made samples and store at recommended temperature until use
Lower/ Higher readings in samples and standards	Not fully thawed kit components	Wait for components to thaw completely and gently mix prior use
	Out-of-date kit or incorrectly stored reagents	Always check expiry date and store kit components as recommended on the datasheet
	Reagents sitting for extended periods on ice	Try to prepare a fresh reaction mix prior to each use
	Incorrect incubation time/ temperature	Refer to datasheet for recommended incubation time and/ or temperature
	Incorrect amounts used	Check pipette is calibrated correctly (always use smallest volume pipette that can pipette entire volume)

Problem	Reason	Solution
Standard curve is not linear	Not fully thawed kit components	Wait for components to thaw completely and gently mix prior use
	Pipetting errors when setting up the standard curve	Try not to pipette too small volumes
	Incorrect pipetting when preparing the reaction mix	Always prepare a master mix
	Air bubbles in wells	Air bubbles will interfere with readings; try to avoid producing air bubbles and always remove bubbles prior to reading plates
	Concentration of standard stock incorrect	Recheck datasheet for recommended concentrations of standard stocks
	Errors in standard curve calculations	Refer to datasheet and re-check the calculations
	Use of other reagents than those provided with the kit	Use fresh components from the same kit

For further technical questions please do not hesitate to contact us by email (technical@abcam.com) or phone (select "*contact us*" on www.abcam.com for the phone number for your region).

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