



Oxaloacetate Assay Kit

Catalog Number KA3793

100 assays

Version: 02

Intended for research use only

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Introduction

Intended Use

- Application
Direct Assays: oxaloacetate in plasma, serum, tissue and culture media.
- Key Features:
Sensitive and accurate: linear detection range: 7 to 400 μM oxaloacetate for colorimetric assays and 1 to 40 μM for fluorimetric assays.

Background

OXALOACETATE (OAA) is an intermediate in the citric acid cycle and participates in gluconeogenesis. OAA is formed by the oxidation of malate, by deamidation of aspartate or by condensation of CO_2 with pyruvate or phosphoenolpyruvate.

Principle of the Assay

The Oxaloacetate Assay Kit provides a simple, direct and automation-ready procedure for measuring oxaloacetate concentration. OAA is converted into pyruvate which is then oxidized with the conversion of the dye into a colored and fluorescent form. The color intensity of the oxidized dye at 570 nm or fluorescence intensity at $\lambda_{\text{ex/em}} = 530/585 \text{ nm}$ is directly proportional to the oxaloacetate concentration in the sample.

General Information

Materials Supplied

List of component

Component	Amount
Developer	10 mL
Dye Reagent	120 µL
ODC Enzyme	120 µL
Oxaloacetate Standard	Dried

Storage Instruction

Store all kit components at -20°C. Shelf life of six months after receipt.

Materials Required but Not Supplied

Pipetting devices, clear or black flat-bottom 96-well plates, plate reader or centrifuge tubes.

Precautions for Use

Reagents are for research use only. Normal precautions for laboratory reagents should be exercised while using the reagents.

Assay Protocol

Sample Preparation

Tissue or cell samples (2×10^6) can be homogenized in 100 μ L PBS. Centrifuge at 14,000 rpm for 5 min. Supernatants should then be deproteinated using a 10 kDa spin filter (e.g. Amicon Ultra-0.5). If assaying serum or plasma, samples must be deproteinated and an internal standard should be used. If planning to measure oxaloacetate in culture media, if possible avoid media with high pyruvate concentrations (e.g. DMEM, L-15, F12, etc.).

Assay Procedure

- Colorimetric Procedure

1. Standards. Dissolve the Oxaloacetate Standard with 100 μ L dH₂O to make a 10 mM stock. Keep standard cold and store at -20°C. Reconstituted OAA standard should be used within 2 weeks. Prepare a 400 μ M Premix by diluting 20 μ L of the 10 mM standard with 480 μ L dH₂O. Next, dilute standards in 1.5 mL centrifuge tubes as follows. If assaying culture media with phenol red, dilute the Oxaloacetate Standard in culture media.

No	Premix + dH ₂ O	Oxaloacetate (μ M)
1	100 μ L + 0 μ L	400
2	60 μ L + 40 μ L	240
3	30 μ L + 70 μ L	120
4	0 μ L + 100 μ L	0

Transfer 20 μ L of each standard to separate wells in a clear flat-bottom 96 well plate.

2. Samples. Add 20 μ L of each sample to two separate wells in a 96 well plate (each sample requires a sample blank).

Samples requiring an internal standard will need three separate reactions: 1) Sample plus standard, 2) Sample alone and 3) Sample Blank. For the internal standard, prepare 500 μ L 80 μ M OAA standard by mixing 100 μ L 400 μ M Premix and 400 μ L dH₂O. For the sample plus standard well, add 5 μ L 80 μ M OAA and 20 μ L sample. For the sample and sample blank wells, add 5 μ L dH₂O and 20 μ L sample.

3. Oxaloacetate Detection. Prepare enough working reagent (WR) for all standards and samples. For each reaction combine the following: 85 μ L Developer, 1 μ L ODC Enzyme and 1 μ L Dye Reagent. For the sample blanks, prepare a WR without the ODC Enzyme. Add 80 μ L of the appropriate WR to each standard and sample well. Mix well and incubate protected from light for 15 min at RT.
4. Read OD_{570nm}.

- Fluorimetric Procedure
 1. For fluorimetric assays, the linear detection range is 1 to 40 μM oxaloacetate. Dilute the standards prepared in Colorimetric Procedure 1:10 in dH_2O . If an internal standard is used, use the same concentration as described in the Colorimetric Procedure (i.e. 5 μL of 80 μM OAA).
 2. Transfer 20 μL standards and 20 μL samples (2 wells per sample if a standard curve is used; 3 wells per sample if an internal standard is used, see Colorimetric Procedure) into separate wells of a black 96-well plate. Add 80 μL of appropriate Working Reagent (see Colorimetric Procedure) to each well. Tap plate to mix.
 3. Incubate 15 min at RT and read fluorescence at $\lambda_{\text{ex/em}} = 530/585 \text{ nm}$.

Data Analysis

Calculation of Results

Subtract the blank value (#4) from the standard values and plot the ΔOD or ΔF against standard concentrations. Determine the slope and calculate the oxaloacetate concentration of the Samples as follows:

$$[\text{Oxaloacetate}] = \frac{R_{\text{SAMPLE}} - R_{\text{BLANK}}}{\text{Slope } (\mu\text{M}^{-1})} \times n (\mu\text{M})$$

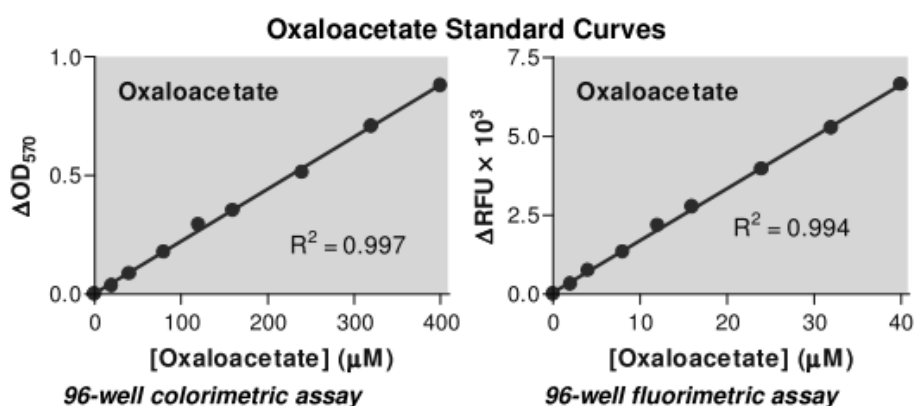
If an internal standard was used, the sample oxaloacetate concentration is computed as follows:

$$[\text{Oxaloacetate}] = \frac{R_{\text{SAMPLE}} - R_{\text{BLANK}}}{R_{\text{STANDARD}} - R_{\text{SAMPLE}}} \times 20 (\mu\text{M})$$

where R_{SAMPLE} , R_{BLANK} , and R_{STANDARD} are optical density or fluorescence intensity readings of the Sample, Sample Blank, and the Sample plus Standard, respectively. n is the sample dilution factor.

Notes: The volume of the internal standard is 4× lower than the sample volume; thus, the sample to standard ratio is multiplied by 20 μM and not 80 μM . If the calculated oxaloacetate concentration is >400 μM for the colorimetric assay, or >40 μM for the fluorimetric assay, dilute sample in dH_2O and repeat assay. Multiply result by the dilution factor n .

Conversions: 100 μM oxaloacetate equals 13.1 mg/L, 0.00131% or 13.1 ppm.



Resources

References

1. Laplante, A et al. (1995) Assay of Blood and Tissue Oxaloacetate and α -Ketoglutarate by Isotope Dilution Gas Chromatography-Mass Spectrometry. Anal. Biochem. 224: 580-7.
2. Parvin, R et al (1980). Convenient rapid determination of picomole amounts of oxaloacetate and oxaloacetate. Anal. Biochem.104: 296-9.
3. Eschenbrenner E, Guynn RW (1976). Measurement of oxaloacetate in tissue extracts by enzymatic cycling. Anal Biochem. 72:220-9.