EnzyChrom™ Oxaloacetate Assay Kit (EOAA-100)

Quantitative Colorimetric/Fluorimetric Oxaloacetate Determination

DESCRIPTION

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. BioAssay Systems' 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 $\lambda_{ex/em}$ = 530/585 nm is directly proportional to the oxaloacetate concentration in the sample.

KEY FEATURES

Sensitive and accurate. Linear detection range: 7 to 400 µM oxaloacetate for colorimetric assays and 1 to 40 µM for fluorimetric assays.

APPLICATIONS

Direct Assays: oxaloacetate in plasma, serum, tissue and culture media.

KIT CONTENTS (100 TESTS IN 96-WELL PLATES)

Developer:	10 mL	**ODC Enzyme:**	120 µL
Dye Reagent:	120 µL	**Oxaloacetate Standard**:	Dried

Storage conditions. The kit is shipped on ice. Store all kit components at -20 °C. Shelf life of six months after receipt.

Precautions: reagents are for research use only. Normal precautions for laboratory reagents should be exercised while using the reagents. Please refer to Material Safety Data Sheet for detailed information.

PROCEDURES

Sample Preparation

Tissue or cell samples (2 x10^6) can be homogenized in 100 µL 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.).

Colorimetric Procedure

1. *Standards*. Dissolve the Oxaloacetate Standard with 100 µL dH_2O 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 µM Premix by diluting 20 µL of the 10 mM standard with 480 µL dH_2O. 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_2O	Oxaloacetate (µM)
1	100 µL + 0 µL	400
2	60 µL + 40 µL	240
3	30 µL + 70 µL	120
4	0 µL + 100 µL	0

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

2. *Samples*. Add 20 µL 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 µL 80 µM OAA standard by mixing 100 µL 400 µM Premix and 400 µL dH_2O. For the sample plus standard well, add 5 µL 80 µM OAA and 20 µL sample. For the sample and sample blank wells, add 5 µL dH_2O and 20 µL sample.

3. *Oxaloacetate Detection*. Prepare enough working reagent (WR) for all standards and samples. For each reaction combine the following: 85 µL Developer, 1 µL ODC Enzyme and 1 µL Dye Reagent. For the sample blanks, prepare a WR ***without*** the ODC Enzyme. Add 80 µL 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

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).

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.

Incubate 15 min at RT and read fluorescence at $\lambda_{ex/em}$ = 530/585 nm.

CALCULATION

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:

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

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

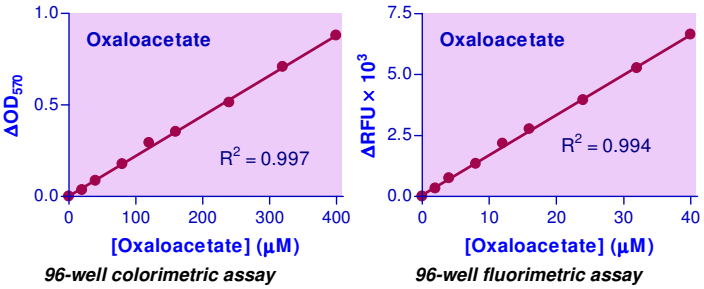
$$[\text{Oxaloacetate}] = \frac{R_{SAMPLE} - R_{BLANK}}{R_{STANDARD} - R_{SAMPLE}} \times 20 \quad (\mu M)$$

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.

Oxaloacetate Standard Curves



96-well colorimetric assay ***96-well fluorimetric assay***

MATERIALS REQUIRED, BUT NOT PROVIDED

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

LITERATURE

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.