Oxaloacetate Assay Kit (Colorimetric)

Oxaloacetate Assay Kit (Colorimetric) is a detection kit for the quantification of Oxaloacetate in serum, plasma, tissue and cell culture supernatants.

Catalog number: ARG82187

Package: 100 tests

For research use only. Not for use in diagnostic procedures.

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INTRODUCTION

Oxaloacetic acid (also known as oxalacetic acid or OAA) is a crystalline organic compound with the chemical formula $HO_2CC(O)CH_2CO_2H$. Oxaloacetic acid, in the form of its conjugate base oxaloacetate, is a metabolic intermediate in many processes that occur in animals. It takes part in gluconeogenesis, the urea cycle, the glyoxylate cycle, amino acid synthesis, fatty acid synthesis and the citric acid cycle. [Provide by Wikipedia: Oxaloacetic acid]

PRINCIPLE OF THE ASSAY

This Oxaloacetate Assay Kit (Colorimetric) is a simple colorimetric assay that measures the amount of Oxaloacetate (OAA) present in various biological samples such as serum, plasma, tissue and cell culture supernatant. 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 O.D. 570 nm or fluorescence intensity at λex/em = 530/585 nm is directly proportional to the oxaloacetate concentration in the sample.

MATERIALS PROVIDED & STORAGE INFORMATION

The kit is shipped on dry ice. Store all components at -20°C upon receiving. Shelf life: 6 months after receipt.

Component	Quantity	Storage information
Developer	10 mL	-20°C
ODC Enzyme	120 µL	-20°C
Dye Reagent	120 µL	-20°C
Standard (Oxaloacetate, lyophilized)	1 vial	-20°C

MATERIALS REQUIRED BUT NOT PROVIDED

- Microplate reader capable of reading at O.D. 570 nm
- Fluorescence microplate reader capable of reading excitation at 530 nm and emission at 585 nm
- Centrifuge and centrifuge tube
- Clear or black flat-bottom 96 well microplate
- Deionized or Distilled water
- Pipettes, pipette tips and Multichannel micropipette reservoir

TECHNICAL NOTES AND PRECAUTIONS

- Wear protective gloves, clothing, eye, and face protection especially while handling blood or body fluid samples.
- If planning to measure oxaloacetate in culture media, if possible avoid media with high pyruvate concentrations (e.g. DMEM, L-15, F12, etc.).
- 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.
- All reagents should be mixed by gentle inversion or swirling prior to use. Do not induce foaming.
- Before using the kit, spin tubes and bring down all components to the bottom of tubes.
- It is highly recommended assaying the Standards and samples in duplicates.
- Change pipette tips between the addition of different reagent or samples.

SAMPLE COLLECTION & STORAGE INFORMATION

The sample collection and storage conditions listed below are intended as general guidelines. Sample stability has not been evaluated.

Serum: Collect blood in a tube with no anticoagulant. Allow the blood to clot at room temperature for 30 minutes. Centrifuge at 2500 x g for 20 minutes at 4°C. Samples must be deproteinated and an internal standard should be used.

Plasma: Collect blood with heparin or citrate and centrifuge at 2000 x g for 10 minutes at 4°C. Samples must be deproteinated and an internal standard should be used.

Tissue or cell lysate: Tissue or cell samples (2 x10^6) can be homogenized in 100 µL of PBS. Centrifuge at 10,000 x g for 5 minutes at 4°C. Supernatants should then be deproteinated using a 10 kDa spin filter (E.g., Amicon Ultra-0.5).

Cell culture supernatant: Avoid culture medium with high pyruvate concentration (E.g., DMEM, L-15, F-12, etc.). Centrifuge at 1000 x g for 10 minutes. Collection the supernatant for assay.

REAGENT PREPARATION

- **Working Reagent:** for each assay, mix 85 µL of Developer, 1 µL of ODC Enzyme and 1 µL of Dye Reagent. Prepare immediately before assay.
- **Blank Working Reagent:** for each assay, mix 85 µL of Developer and 1 µL of Dye Reagent (without the ODC Enzyme). Prepare immediately before assay.
- **Standards:** Dissolve the Oxaloacetate Standard with 100 µL of distilled water to make a 10 mM stock. Store Standard 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 of distilled water. Dilute standards as follows.

Standard tube	Oxaloacetate (µM)	Distilled water (µL)	Standard Premix, 400 µM (µL)
S1	400	0	100
S2	240	40	60
S3	120	70	30
S4	0	100	0

ASSAY PROCEDURE

Equilibrate all components to room temperature. Briefly centrifuge the tubes before opening.

COLORIEMTRIC PROCEDURE

	Standard well	Sample well	Blank well
Each diluted Standard	20 µL		
Each Sample		20 µL	20 µL
Working Reagent	80 µL	80 µL	
Blank Working Reagent			80 µL
Tap plate to mix briefly and thoroughly. Incubate for **15 minutes** at **room temperature** in the dark.			
Read the absorbance at **O.D. 570 nm**.			

Note: If using an internal standard, samples will need three separate reactions: 1) sample plus standard, 2) sample alone and 3) sample blank. For the internal standard, prepare 500 µL of 80 µM OAA standard by mixing 100 µL of 400 µM Premix and 400 µL of distilled water. For the sample plus standard well, add 5 µL of 80 µM OAA and 20 µL of sample.

FLUORIMETRIC PROCEDURE

Follow COLORIEMTRIC PROCEDURE with the change below. The linear detection range is 1 to 40 µM of Oxaloacetate. Dilute the standards prepared in Colorimetric Procedure 1:10 in distilled water. If an internal standard is used, use the same concentration as described in the Colorimetric Procedure (E.g., 5 µL of 80 µM OAA). Use black flat-bottom 96 well microplate for assay. And read the fluorescence at λex/em = 530/585 nm.

CALCULATION OF RESULTS

1. Subtract blank value (distilled water, S4) from the standard values and plot the ΔOD or ΔRFU against standard concentrations. Determine the slope and calculate the oxaloacetate concentration of Sample as follows:

   Oxaloacetate (µM) = [(R_{Sample} – R_{Blank}) / Slope (μM^{-1})] x n

2. If an internal standard was used, the sample maltose concentration is computed as follows:

   Oxaloacetate (µM) = [(R_{Sample} – R_{Blank}) / ($R_{Standard}$ – R_{Sample})] x 20

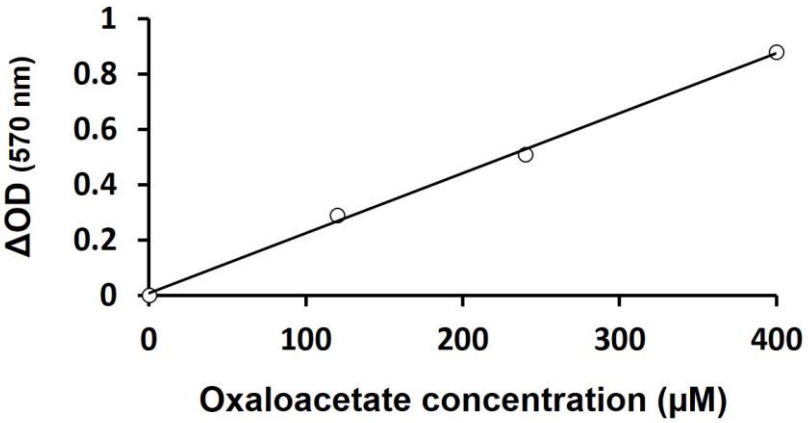
   Note:

   - R_{Sample}, R_{Blank} and $R_{Standard}$: the O.D. 570 nm values and fluorescence intensity values of the sample and sample blank and sample plus Standard, respectively.
   - n is the sample dilution factor.
   - The volume of the internal standard is 4x lower than the sample volume; the sample to standard ratio is multiplied by 20 µM and not 80 µM.

2. Conversions: 100 µM oxaloacetate equals 13.1 mg/L, 0.00131% or 13.1 ppm.
3. If the calculated oxaloacetate concentration is >400 µM for the colorimetric assay, or >40 µM for the fluorimetric assay, dilute sample in distilled water and repeat assay. Multiply result by the dilution factor n.

EXAMPLE OF TYPICAL STANDARD CURVE

The following figures demonstrate typical results with the Oxaloacetate Assay Kit (Colorimetric). One should use the data below for reference only. This data should not be used to interpret actual results.



QUALITY ASSURANCE

Sensitivity

OD: 4 μM; FL: 1 μM