Oxaloacetate

Colorimetric Microplate Assay Kit

User Manual

Catalog # CAK1284

(Version 1.1A)

Detection and Quantification of Oxaloacetate (OAA) Content in Urine, Serum, Plasma, Tissue extracts, Cell lysate, Cell culture media and Other biological fluids Samples.

For research use only. Not for diagnostic or therapeutic procedures.

I. INTRODUCTION

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 CO2 with pyruvate or phosphoenolpyruvate. Oxaloacetate Colorimetric Microplate Assay Kit provides a simple procedure for measuring oxaloacetate concentration. OAA is converted into pyruvate which is then oxidized with the conversion of the dye into a colored form, and can be measured at a colorimetric readout at 550 nm.

II. KIT COMPONENTS

Component	Volume	Storage
96-Well Microplate	1 plate	
Assay Buffer	30 ml x 4	4 °C
Reaction Buffer	20 ml x 1	4 °C
Enzyme	Powder x 1	-20 °C
Dye Reagent	Powder x 1	-20 °C
Standard Diluent	10 ml x 1	4 °C
Standard	Powder x 1	4 °C
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III. MATERIALS REQUIRED BUT NOT PROVIDED

1. Microplate reader to read absorbance at 550 nm
2. Distilled water
3. Pipettor, multi-channel pipettor
4. Pipette tips
5. Mortar
6. Ice
7. Centrifuge
8. Timer

IV. REAGENT PREPARATION

Standard: Briefly centrifuge prior to opening. Dissolve in 0.5 ml Standard Diluent to generate 50 mmol/L of standard stock solution, keep in dark and store at 4 °C for 1-2 weeks or -20 °C for 1 month after reconstitution. Then dilute to 5 mmol/L standard top solution by adding 50 µl stock solution into 450 µl Standard Diluent. Perform 2-fold serial dilutions of the top standard solution using Standard Diluent to make the standard curve. The concentration of standard curve could be 5/2.5/1.25/0.625/0.312/0.156/0.078 mmol/L.

Enzyme: Add 3 ml Reaction Buffer to dissolve before use. keep in dark and store at 4 °C for 1 week or -20 °C for 1-2 months after reconstitution.

Dye Reagent: Add 3 ml Reaction Buffer to dissolve before use. keep in dark and store at 4 °C for 1 week or -20 °C for 1-2 months after reconstitution.

Note: Divide into small aliquots to avoid repeated freeze-thaw cycles

V. SAMPLE PREPARATION

1. For cell and bacteria samples

Collect cell or bacteria into centrifuge tube, discard the supernatant after centrifugation, add 0.1 ml Assay buffer for 5×10^6 cell or bacteria, sonicate (with power 20%, sonicate 3s, interval 10s, repeat 30 times); centrifuged at 12000g 4 °C for 10 minutes, supernatants should then be deproteinated using a 3 kDa spin filter (e.g. Amicon Ultra-0.5).

2. For tissue samples

Weigh 0.1 g tissue, homogenize with 0.1 ml Assay buffer on ice, centrifuged at 12000g 4 °C for 10 minutes, supernatants should then be deproteinated using a 3 kDa spin filter (e.g. Amicon Ultra-0.5).

3. For liquid samples

If assaying serum or plasma, samples must be deproteinated. If planning to measure oxaloacetate in culture media. Avoid media with high pyruvate concentrations (e.g. DMEM, L-15, F12, etc.).

V. ASSAY PROCEDURE

Add following reagents into the microplate:

Reagent*	Sample**	Standard	Blank
Reaction Buffer	130 µl	130 µl	130 µl
Sample	10 µl	--	--
Standard	--	10 µl	--
Distilled water	--	--	10 µl
Enzyme	30 µl	30 µl	30 µl
Dye Reagent	30 µl	30 µl	30 µl
Mix and incubate at 37 °C for 20 minutes. Then record absorbance measured at 550 nm.			

Note:

*Reagents must be added sequentially and should not be premixed prior to addition.

** The concentrations can vary over a wide range depending on the different samples. For unknown samples, we recommend doing a pilot experiment & testing several doses to ensure the readings are within the standard curve range.

**For colored samples, we recommend setting a parallel sample background control well with same volume of sample only. Other reagents were replaced by distilled water to the same total volume. Subtract the OD value of the sample background control from the OD value of the sample to correct for interference from the sample's own color.

VII. CALCULATION

1. Calculate the sample concentration in ASSAY PROCEDURE according to the slope of the standard curve

$$C = \frac{(OD_{Sample} - OD_{Blank}) - Intercept}{Slope} \times n \ (mmol/L)$$

Calculate the initial concentration according to sample preparation procedure.

2. According to one point of the standard OD and concentration

2.1 According to the quantity of cells or bacteria

$$C = \frac{(C_{Standard} \times V_{Standard}) \times (OD_{Sample} - OD_{Blank})}{(OD_{Standard} - OD_{Blank}) \times N \times (V_{Sample} / V_{Assay})} \ (\mu mol/10^4)$$

2.2 According to the weight of sample

$$C = \frac{(C_{Standard} \times V_{Standard}) \times (OD_{Sample} - OD_{Blank})}{(OD_{Standard} - OD_{Blank}) \times W \times (V_{Sample} / V_{Assay})} \ (\mu mol/g)$$

2.3 According to the volume of sample

$$C = \frac{(C_{Standard} \times V_{Standard}) \times (OD_{Sample} - OD_{Blank})}{(OD_{Standard} - OD_{Blank}) \times V_{Sample}} \ (\mu mol/ml)$$

Slope: the absorbance slope of standard curve

n: the dilution factor

$C_{Standard}$: the standard concentration, mmol/L = μmol/ml

$V_{Standard}$: the volume of standard in assay procedure, μl

V_{Sample}: the volume of sample in assay procedure, μl

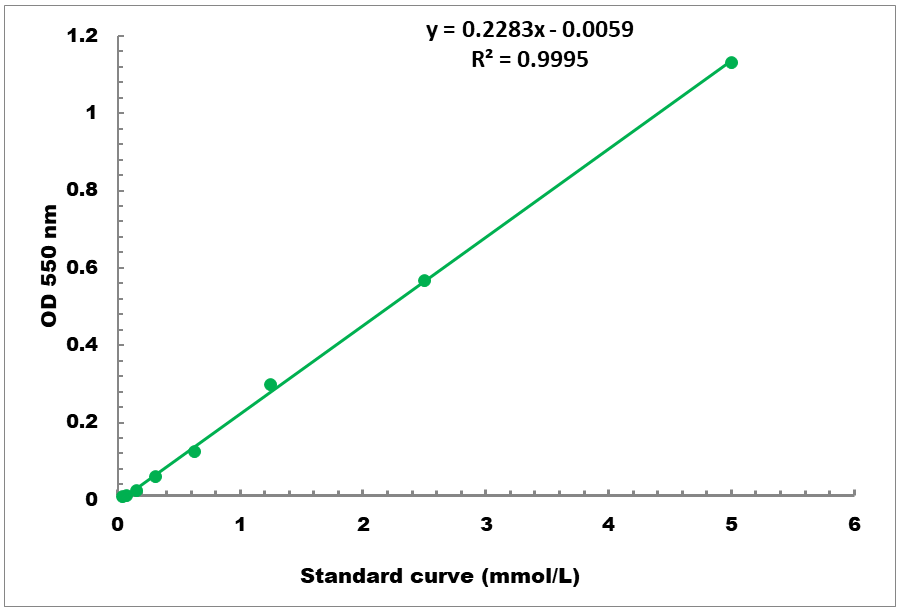
V_{Assay}: the volume of Assay Buffer, μl

W: the weight of sample, g

N: the quantity of cell or bacteria, 10^4

VIII. TYPICAL DATA

The standard curve is for demonstration only. A standard curve must be run with each assay.



Detection Range: 0.05 mmol/L - 5 mmol/L