



Citrate Lyase Activity Colorimetric Microplate Assay Kit User Manual

Catalog # CAK1321

(Version 1.1A)

Detection and Quantification of Citrate Lyase Activity in Bacteria
lysate and Plant tissue Sample.

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

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I. INTRODUCTION

Citrate lyase is a coenzyme A-dependent intracellular enzyme primarily found in bacteria and plants. It cleaves citrate into oxaloacetate and acetate, participating in microbial citrate metabolism. Unlike ATP-citrate lyase in animal cells, it does not consume ATP and yields acetate rather than acetyl-CoA, and is therefore not involved in lipid biosynthesis. Measuring citrate lyase activity is useful for assessing microbial metabolic capacity and is commonly applied in food fermentation, industrial biotechnology, and plant metabolism research.

Citrate lyase Activity Colorimetric Microplate Assay Kit provides a convenient tool for sensitive detection of citrate lyase activity in a variety of samples. Citrate cleaves citrate into oxaloacetate and acetate. NADH is generated through a linked enzymatic reaction, which reacts with dye reagent to form a colored product. The color intensity reduction at 450nm is proportional to the Citrate lyase activity in the sample.

II. KIT COMPONENTS

Component	Volume	Storage
96-Well Microplate	1 plate	
Assay Buffer	30 ml x 4	4 °C
Reaction Buffer	5 ml x 1	4 °C
Substrate	1 ml x 1	4 °C
Diluent	1 ml x 1	4 °C
Coenzyme	Powder x 1	4 °C, keep in dark
Enzyme Diluent	1.2 ml x 1	4 °C
Enzyme	Powder x 1	-20 °C
Dye Reagent A	Powder x 1	4 °C, keep in dark
Dye Reagent B	1 ml x 1	4 °C, keep in dark
Standard	Powder x 1	4 °C
Positive Control	Powder x 1	-20 °C
Technical Manual	1 Manual	

III. MATERIALS REQUIRED BUT NOT PROVIDED

1. Microplate reader to read absorbance at 450 nm
2. Distilled water
3. Pipettor, multi-channel pipettor
4. Pipette tips
5. Mortar
6. Centrifuge
7. Timer

IV. REAGENT PREPARATION

Standard: Briefly centrifuge prior to opening. Dissolve in 1 ml distilled water to generate 2 mmol/L of standard stock solution, store at 4 °C for 1 day or -20°C for 3 days after reconstitution. Then dilute to 400 µmol/L standard top solution by adding 0.2 ml stock solution into 0.8 ml distilled water. Perform 2-fold serial dilutions of the top standard solution using distilled water to make the standard curve. The concentration of standard curve could be 400/200/100/50/25/12.5/6.25 µmol/L.

Coenzyme: Briefly centrifuge prior to opening. Add 1 ml Diluent to dissolve before use. Immediately prepare before use of store at -20°C for 2-3 weeks.

Enzyme: Briefly centrifuge prior to opening. Add 1 ml Enzyme Diluent to dissolve before use. Store at 4°C for 1-2 days or -20°C for 4-5 months.

Dye Reagent A: Add 10 ml distilled water to dissolve before use, mix. Keep in dark and store at 4°C for 1 week or -20°C for 1 month.

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

V. SAMPLE PREPARATION

1. For bacteria samples

Collect bacteria into centrifuge tube, discard the supernatant after centrifugation, add 1 ml Assay Buffer for 5×10^6 bacteria (the quantity should be adjusted according to the actual situation of the sample), sonicate (with power 20%, sonicate 3s, interval 10s, repeat 30 times); centrifuged at 10000g 4 °C for 10 minutes, take the supernatant into a new centrifuge tube and keep it on ice for detection.

2. For tissue samples

Weigh out 0.1 g tissue (the quantity should be adjusted according to the actual situation of the sample), homogenize with 1 ml Assay Buffer on ice, centrifuged at 10000g 4 °C for 10 minutes, take the supernatant into a new centrifuge tube and keep it on ice for detection.

VI. ASSAY PROCEDURE

Add following reagents in the microplate:

Reagent*	Sample**	Control	Positive Control	Standard	Blank
Reaction Buffer	50 μ l	50 μ l	50 μ l	--	--
Sample	10 μ l	--	--	--	--
Distilled water	--	10 μ l	--		90 μ l
Positive control	--	--	10 μ l	--	--
Substrate	10 μ l	10 μ l	10 μ l	--	--
Coenzyme	10 μ l	10 μ l	10 μ l	--	--
Enzyme	10 μ l	10 μ l	10 μ l	--	--
Mix and put in the oven at 37 °C for 15 minutes					
Standard	--	--	--	90 μ l	--
Dye Reagent A	100 μ l	100 μ l	100 μ l	100 μ l	100 μ l
Dye Reagent B	10 μ l	10 μ l	10 μ l	10 μ l	10 μ l
Mix, keep at RT for 2 minutes, measured at 450 nm and record the absorbance.					

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

Unit Definition: One unit of Citrate lyase activity is defined as the enzyme oxidizes 1 μmol of NADH per minute.

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

$$C = \frac{(\text{OD}_{\text{Control}} - \text{OD}_{\text{Sample}}) - \text{Intercept}}{\text{Slope}} \times \frac{V_{\text{Standard}}}{V_{\text{Sample}}} \times n \text{ (U/mL)}$$

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 bacteria

$$\text{Activity} = \frac{(C_{\text{Standard}} \times V_{\text{Standard}}) \times (\text{OD}_{\text{Control}} - \text{OD}_{\text{Sample}})}{(\text{OD}_{\text{Standard}} - \text{OD}_{\text{Blank}}) \times N \times (V_{\text{Sample}} / V_{\text{Assay}}) \times T} \text{ (U/10}^4\text{)}$$

2.3 According to the weight of sample

$$\text{Activity} = \frac{(C_{\text{Standard}} \times V_{\text{Standard}}) \times (\text{OD}_{\text{Control}} - \text{OD}_{\text{Sample}})}{(\text{OD}_{\text{Standard}} - \text{OD}_{\text{Blank}}) \times W \times (V_{\text{Sample}} / V_{\text{Assay}}) \times T} \text{ (U/g)}$$

Slope: the absorbance slope of standard curve

n: the dilution factor

C_{Standard} : the standard concentration, $\text{mmol/L} = \mu\text{mol/ml}$

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

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

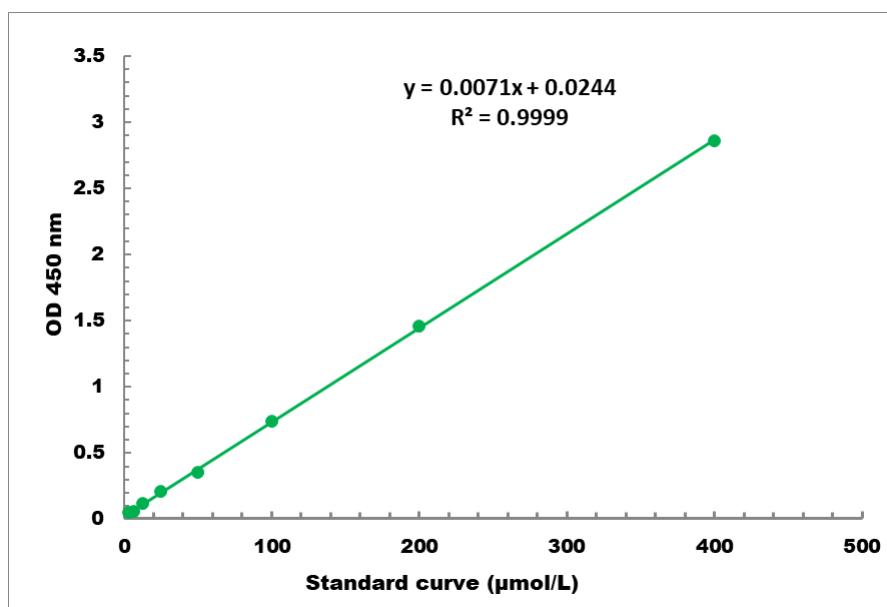
V_{Assay} : the volume of Assay Buffer, μl

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

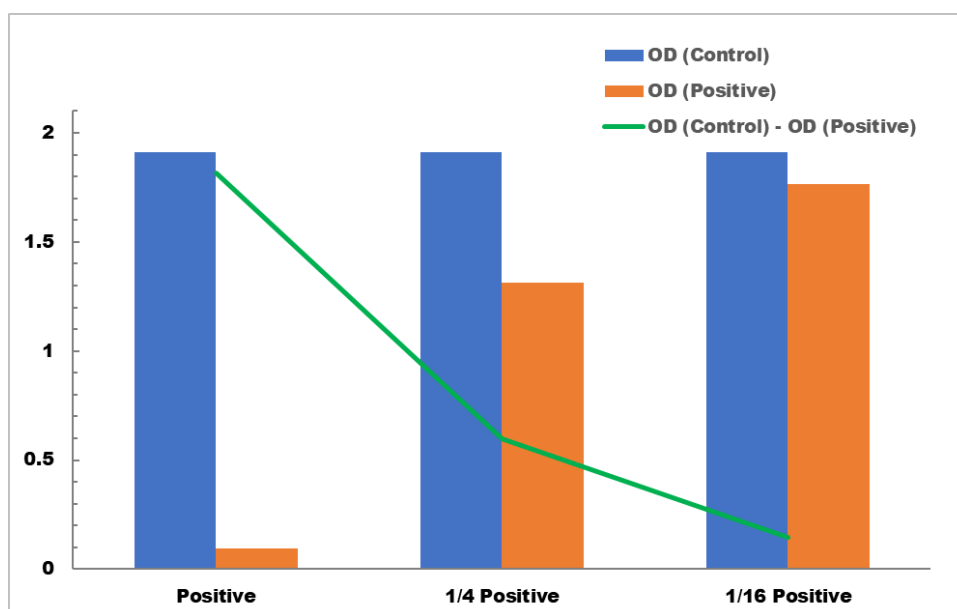
W: the weight of sample, g

VIII. TYPICAL DATA

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



Detection Range: 4 μmol/L - 400 μmol/L



Positive Control reaction in 96-well plate assay with decreasing the concentration