Citrate Synthase Activity

Colorimetric Microplate Assay Kit

User Manual

Catalog # CAK1119

(Version 1.2A)

Detection and Quantification of Citrate Synthetase (CS) Activity in Tissue extracts, Cell lysate Samples and Other biological fluids.

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

I. INTRODUCTION

Citrate synthase (EC 2.3.3.1) is the initial enzyme of the tricarboxylic acid (TCA) cycle. This enzyme is a 51.7 kDa enzyme that catalyzes the reaction of 2 carbon acetyl CoA with 4 carbon oxaloacetate to form the 6 carbon citrate. This enzyme is an exclusive marker of the mitochondrial matrix. Citrate synthase is found in nearly all cells capable of oxidative metabolism.

Citrate Synthase Activity Colorimetric Microplate Assay Kit is a sensitive assay for determining Citrate Synthase activity in various samples. The activity is determined by recording color development of TNB, which is generated from DTNB present in the reaction of citrate synthesis.

II. KIT COMPONENTS

Component	Volume	Storage
96-Well Microplate	1 plate	
Assay Buffer	30 ml x 4	4 °C
Reaction Buffer	10 ml x 1	4 °C
Diluent A	5 ml x 1	4 °C
Diluent B	15 ml x 1	4 °C
Coenzyme	Powder x 1	-20 °C
Substrate	Powder x 1	-20 °C
Dye Reagent	Powder x 1	4 °C
Standard	Powder x 1	4 °C
Positive Control	Powder x 1	-20 °C
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Note: Avoid freeze-thaw cycles

III. MATERIALS REQUIRED BUT NOT PROVIDED

1. Microplate reader to read absorbance at 412 nm
2. Distilled water
3. Pipettor, multi-channel pipettor
4. Pipette tips
5. Mortar
6. Centrifuge
7. Timer
8. Ice

IV. REAGENT PREPARATION

Standard: Briefly centrifuge prior to opening. Dissolve in 0.5 ml Diluent B to generate 5 mmol/L of standard stock solution, keep in dark and store at 4 °C for 1 day or -20 °C for 1 month after reconstitution. Then dilute to 500 µmol/L standard top solution by adding 100 µl stock solution into 900 µl Diluent B. Perform 2-fold serial dilutions of the top standard solution using Diluent B to make the standard curve. The concentration of standard curve could be 500/250/125/62.5/31.2/15.6/7.8 µmol/L.

Coenzyme: Briefly centrifuge prior to opening. Dissolve in 1 ml Diluent A before use. Keep in dark and store at 4 °C for 1 day or -20 °C for 2 weeks.

Substrate Working Solution: Briefly centrifuge Substrate prior to opening. Dissolve in 0.15 ml Diluent A to generate Substrate (10X) stock solution. Keep in dark and store at 4 °C for 1 day or -20 °C for 2 weeks. Then dilute to Substrate Working Solution by adding 100 µl stock solution into 900 µl Diluent A.

Dye Reagent: Add 3 ml Diluent B to dissolve before use. Keep in dark and store at 4 °C for 1 week or -20 °C for 1 month.

V. SAMPLE PREPARATION

1. For cell and bacteria samples

Collect cell or bacteria into centrifuge tube, discard the supernatant after centrifugation, add 1 ml Assay Buffer for 5×10^6 cell or 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.

V. ASSAY PROCEDURE

Add following reagents into the microplate:

Reagent*	Sample**	Control	Positive Control	Standard	Blank
Reaction Buffer	70 µl	70 µl	70 µl	70 µl	70 µl
Sample	10 µl	--	--	--	--
Distilled water	70 µl	80 µl	70 µl	--	100 µl
Positive control	--	--	10 µl	--	--
Coenzyme	10 µl	10 µl	10 µl	--	--
Standard	--	--	--	100 µl	--
Dye Reagent	30 µl	30 µl	30 µl	30 µl	30 µl
Substrate	10 µl	10 µl	10 µl	--	--
Mix and wait for 2 minutes, record absorbance measured at 412 nm.					

Note:

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

**For unknown samples, we recommend doing a pilot experiment & testing several doses to ensure the readings are within the standard curve range. If the enzyme activity is lower, please add more samples into the reaction system; or increase the reaction time; if the enzyme activity is higher, please dilute the sample, or decrease the reaction time.

**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.

VI. CALCULATION

Unit Definition: One unit of CS activity is defined as the enzyme generates 1 μmol of TNB per minute.

1. According to the slope of the standard curve

$$\text{Activity} = \frac{(OD_{Sample} - OD_{Control}) - \text{Intercept}}{\text{Slope} \times T} \times \frac{V_{Standard}}{V_{Sample}} \times n \quad (U/mL)$$

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

2.1 According to the quantity of cells or bacteria

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

2.2 According to the weight of sample

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

Slope: the absorbance slope of standard curve

n: the dilution factor

W: the weight of total sample, g

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

$C_{Standard}$: the concentration of standard, μmol/mL

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

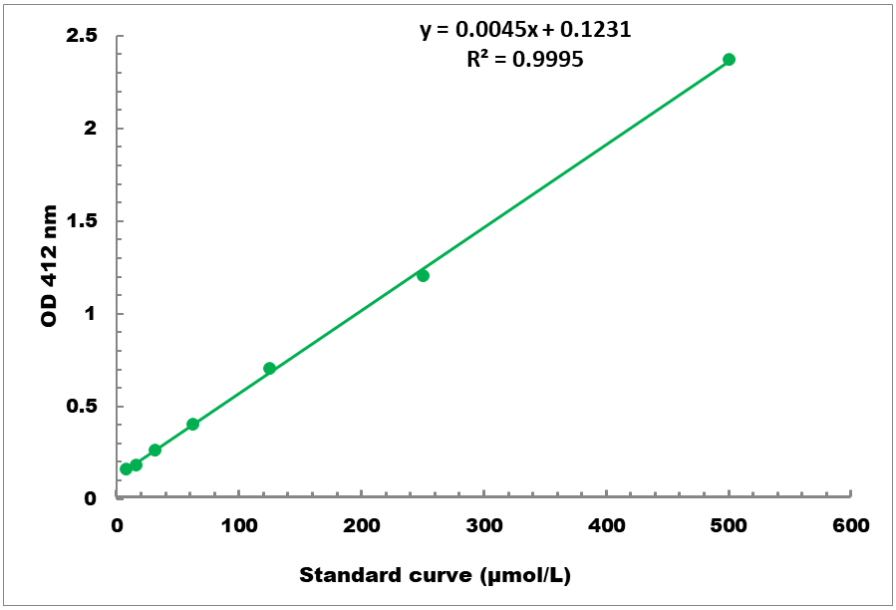
V_{Sample}: the volume of sample in assay procedure, mL

V_{Assay}: the volume of Assay Buffer in sample preparation, mL

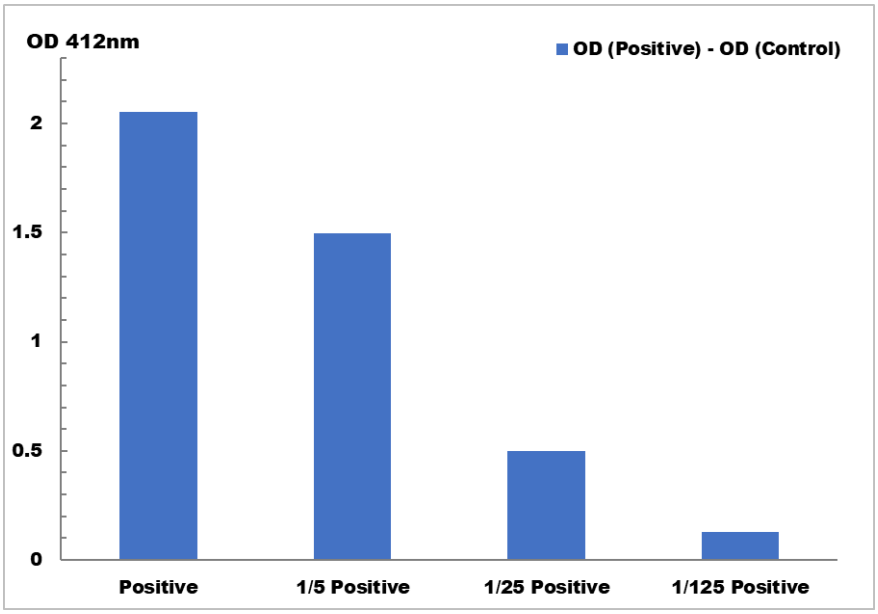
T: the reaction time, minute

VIII. TYPICAL DATA

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



Detection Range: 5 µmol/L - 500 µmol/L



Positive Control reaction with decreasing the concentration