PRODUCT INFORMATION & MANUAL

CS Citrate Synthase Activity Assay Kit (Colorimetric)

NBP3-24481

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

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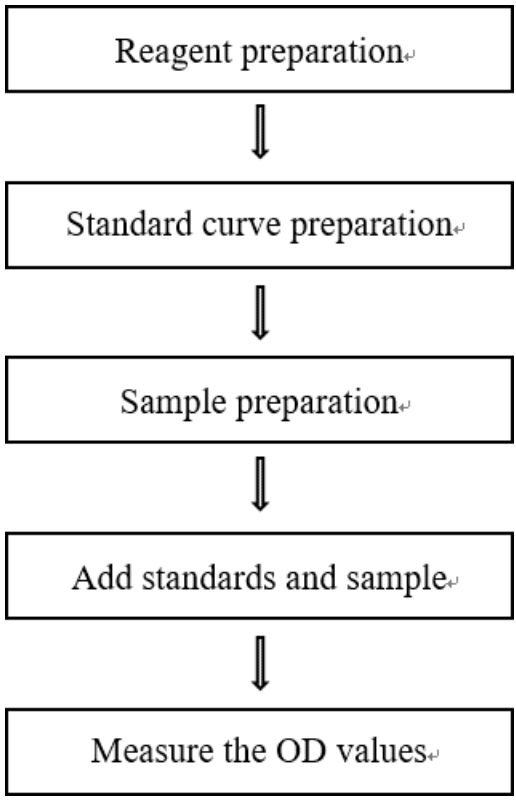
Assay summary



Intended use

This kit can measure citrate synthase (CS) activity in animal, plant tissue and cell samples.

Detection principle

Citrate synthetase (CS) catalyzes acetyl-CoA and oxaloacetate to produce limonyl-CoA, which can further produce CoA. The yellow product generated by this reaction has a characteristic absorption peak at 412 nm. The activity of CS can be calculated by measuring the change of absorbance value at 412 nm.

Kit components & storage

Item	Component	Size 1(48 T)	Size 2(96 T)	Storage
Reagent 1	Extraction Solution	50 mL ×1 vial	50 mL ×2 vials	-20°C, 12 months
Reagent 2	Buffer Solution	9 mL ×1 vial	18 mL ×1 vial	-20°C, 12 months
Reagent 3	Substrate	1.8 mL ×1 vial	1.8 mL ×2 vials	-20°C, 12 months shading light
Reagent 4	Chromogenic Agent	1.6 mL ×1 vial	1.6 mL ×2 vials	-20°C, 12 months shading light
Reagent 5	Standard	Powder ×1 vial	Powder ×2 vials	-20°C, 12 months shading light
	Microplate	96 wells		No requirement
	Plate Sealer	2 pieces		

Note: The reagents must be stored strictly according to the preservation conditions in the above table. The reagents in different kits cannot be mixed with each other. For a small volume of reagents, please centrifuge before use, so as not to obtain sufficient amount of reagents.

Materials prepared by users

Instruments:

Incubator, centrifuge, Microplate reader (402-422 nm, optimum wavelength: 450 nm)

Reagent preparation

① After melting, put the chromogenic agent on ice box for use but no more than for 2 h, and put it back immediately at -20℃ after use. Equilibrate all reagents to room temperature before use.

② The preparation of 10 mmol/L standard solution：
Dissolve a vial of standard with 1 mL double distilled water and mix fully. Aliquot 10 mmol/L standard solution and storage at -20°C for 3 days protected from light, and avoid repeated freeze/thaw cycles is advised.

③ The preparation of 1 mmol/L standard solution：
Prepare 1000 μL of 1 mmol/L standard solution (mix well 100 μL of 10 mmol/L standard solution, 900 μL of double distilled water). The 1 mmol/L standard solution should be prepared on spot, and use up within 2 h.

④ The preparation of standard curve：
Always prepare a fresh set of standards. Discard working standard dilutions after use.
Dilute 1 mmol/L standard solution with double distilled water to a serial concentration. The recommended dilution gradient is as follows: 0, 0.2, 0.3, 0.4, 0.6, 0.8, 0.9, 1 mmol/L. Reference is as follows:

Item	①	②	③	④	⑤	⑥	⑦	⑧
Concentration (mmol/L)	**0**	**0.2**	**0.3**	**0.4**	**0.6**	**0.8**	**0.9**	**1**
1 mmol/L Standard solution (μL)	0	40	60	80	120	160	180	200
Double distilled water (μL)	200	160	140	120	80	40	20	0

Sample preparation

① Sample preparation

Tissue sample:

① Harvest the amount of tissue needed for each assay (initial recommendation 20 mg).

② Homogenize 20 mg tissue in 180 μL extracting solution with a dounce homogenizer at 4°C.

③ Centrifuge at 10000×g for 15 minutes to remove insoluble material. Collect supernatant and keep it on ice for detection.

④ Meanwhile, determine the protein concentration of supernatant.

Cells:

① Harvest the number of cells needed for each assay (initial recommendation 1×10^6 cells).

② Wash cells with PBS (0.01 M, pH 7.4).

③ Homogenize 1×10^6 cells in 200 μL extracting solution with a ultrasonic cell disruptor at 4°C.

④ Centrifuge at 10000×g for 10 minutes to remove insoluble material. Collect supernatant and keep it on ice for detection.

⑤ Meanwhile, determine the protein concentration of supernatant.

② Dilution of sample

The recommended dilution factor for different samples is as follows (for reference only):

Sample type	Dilution factor
10% Rat kidney tissue homogenate	1
10% Rat spleen tissue homogenate	1
10% Rat liver tissue homogenate	1
10% Rat heart tissue homogenate	1
10% Mouse liver tissue homogenate	1
10% Mouse lung tissue homogenate	1

Note: The diluent is extracting solution. For the dilution of other sample types, please do pretest to confirm the dilution factor.

The key points of the assay

① Avoid bubbles when adding reaction working solution.

② After melting, put the reagent 4 on ice box for use but no more than for 2 h, and put it back immediately at -20℃ after use.

Operating steps

① Add 125 μL of buffer solution to standard well and sample well.

② Add 30 μL of substrate to each well..

③ Add 20 μL of chromogenic agent to each well.

④ Mix fully with microplate reader for 3 s, incubate at 37°C for 3 min.

⑤ Standard well: Add 10 μL of standard solution with different concentrations to the corresponding wells.
Sample well: Add 10 μL of sample to the corresponding wells.

⑥ Mix fully with microplate reader for 3 s, and measure the OD value of each well at 412 nm with microplate reader, record as A_1.

⑦ Incubate at 37°C for 8 min.

⑧ Mix fully with microplate reader for 3 s, and measure the OD value of each well at 412 nm with microplate reader, record as A_2, $\triangle A_{412} = A_2 - A_1$.(Plot the standard curve by A_2 value)

Calculation

The standard curve:

1. Average the duplicate reading for each standard.

2. Subtract the mean OD value of the blank (Standard #①) from all standard readings. This is the absoluted OD value.

3. Plot the standard curve by using absoluted OD value of standard and correspondent concentration as y-axis and x-axis respectively. Create the standard curve (**y = ax + b**) with graph software (or EXCEL).

The sample:

1. Tissue sample and cells sample:

Definition: The amount of CS in 1 g tissue or cell protein per 1 minute that produce 1 μmol CoA at 37 ℃ is defined as 1 unit.

$$\text{CS activity (U/gprot)} = (\Delta A_{412} - b) \div a \div T \times 1000 \div C \times f$$

[Note]

ΔA_{412}: The change OD value of sample well.

T: The time of incubation reaction, 8 min

1000*：1 mmol/L = 1000 μmol/L.

C_{pr}: The concentration of protein in sample, gprot/L.

f: Dilution factor of sample before test.

Appendix I Performance Characteristics

1. Parameter:

Intra-assay Precision

Three human serum samples were assayed in replicates of 20 to determine precision within an assay. (CV = Coefficient of Variation)

Parameters	Sample 1	Sample 2	Sample 3
Mean (U/L)	10.5	55.6	87.6
%CV	3.9	5.6	5.5

Inter-assay Precision

Three human serum samples were assayed 17 times in duplicate by three operators to determine precision between assays.

Parameters	Sample 1	Sample 2	Sample 3
Mean (UL)	10.5	55.6	87.6
%CV	6.4	7.5	9.8

Recovery

Take three samples of high concentration, middle concentration and low concentration to test the samples of each concentration for 6 times parallelly to get the average recovery rate of 104%.

	Standard 1	Standard 2	Standard 3
Expected Conc. (mmol/L)	0.2	0.4	0.8
Observed Conc. (mmol/L)	0.2	0.4	0.9
Recovery rate (%)	98	106	108

Sensitivity

The analytical sensitivity of the assay is 2.09 U/L. This was determined by adding two standard deviations to the mean O.D. obtained when the zero standard was assayed 20 times, and calculating the corresponding concentration.

Specification：48T(32 samples)/96T(80 samples)

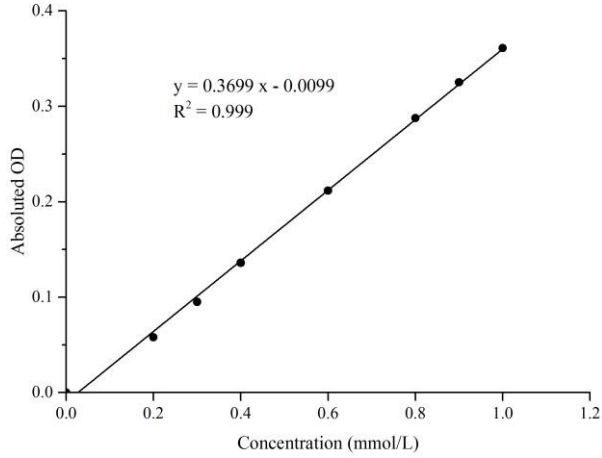
Measuring instrument：Microplate reader（402-422 nm）

Detection:range：2.09–110 U/L

2. Standard curve:

As the OD value of the standard curve may vary according to the conditions of the actual assay performance (e.g. operator, pipetting technique or temperature effects), so the standard curve and data are provided as below for reference only：

Concentration (mmol/L)	0.0	0.2	0.3	0.4	0.6	0.8	0.9	1
Average OD	0.151	0.209	0.246	0.287	0.363	0.439	0.476	0.512
Absoluted OD	0.000	0.058	0.095	0.136	0.212	0.288	0.325	0.361



Appendix Π Example Analysis

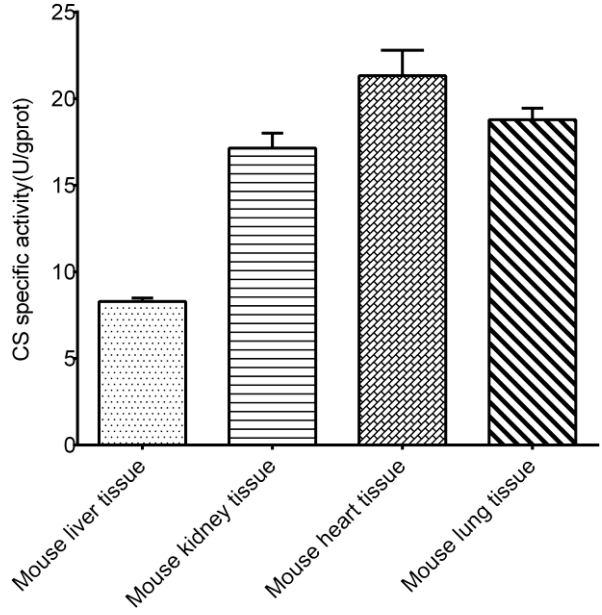
Example analysis：

For mouse liver tissue, take 10 μL of 10% mouse liver tissue homogenate, and carry the assay according to the operation table. The results are as follows:

standard curve: y = 0.3699 x - 0.0099, the OD value of the sample(A_1) is 0.695, the OD value of the sample (A_2) is 0.871, $\Delta A_{412} = A_2 - A_1 = 0.871 - 0.695 = 0.176$, the concentration of protein in sample is 7.40 gprot/L, and the calculation result is:

CS activity (U/gprot) = (0.871 – 0.695 + 0.0099) ÷0.3699 ÷8 ×1000 ÷7.40 = 8.49 U/gprot

Detect 10% mouse liver tissue homogenate (the concentration of protein is 7.40 gprot/L), 10% mouse kidney tissue homogenate (the concentration of protein is 5.36 gprot/L), 10% mouse heart tissue homogenate (the concentration of protein is 3.73 gprot/L) and 10% mouse lung tissue homogenate (the concentration of protein is 2.57 gprot/L) according to the protocol, the result is as follows：



Statement

1. This assay kit is for Research Use Only. We will not response for any arising problems or legal responsibilities causing by using the kit for clinical diagnosis or other purpose.
2. Please read the instructions carefully and adjust the instruments before the experiments. Please follow the instructions strictly during the experiments.
3. Protection methods must be taken by wearing lab coat and latex gloves.
4. If the concentration of substance is not within the detection range exactly, an extra dilution or concentration should be taken for the sample.
5. It is recommended to take a pre-test if your sample is not listed in the instruction book.
6. The experimental results are closely related to the situation of reagents, operations, environment and so on. Novus Biologicals will guarantee the quality of the kits only, and NOT be responsible for the sample consumption caused by using the assay kits. It is better to calculate the possible usage of sample and reserve sufficient samples before use.