Citrate Synthase (CS)Assay Kit

Note: Take two or three different samples for prediction before test.

Detection equipment: Spectrophotometer/Microplate reader

Cat No:NA0551

Size:100T/48S

Components:

Extract solution:50 mL×1, store at -20℃.

Reagent I:10 mL×1, store at -20℃.

Reagent II:0.6 mL×1, store at -20℃.

Reagent III: 40 mL×1, store at 4℃.

Reagent IV: 2 mL×1, store at 4℃.

Reagent V: Powder×2, store -20℃. Add 500 μL of distilled waterwhen the solution will be used. It is suggested that the inexhaustible reagents should still be storage at -20℃.

Reagent VI: Powder×1, store at -20℃. Add 1.5 mL of distilled water when the solution will be used. It is suggested that the inexhaustible reagents should still be storage at -20℃.

Description:

Citrate Synthase (CS,EC 2.3.3.1) is widely exists in animals, plants, microorganism and mitochondrial matrix of cultured cells. It is the first rate-limiting enzyme in the tricarboxylic acid cycle and one of the main regulatory sites.

CS catalyzes acetyl CoA and acetoacetic acid to generate citryl coenzyme A, further hydrolysis to produce citric acid. The reaction promoted the transformation of colorless DTNB to yellow TNB, which has absorption at 412 nm.

Required but not provided

Spectrophotometer/Microplate reader, low temperature centrifuge, water-bath, mortar/homogenizer,adjustablepipette, micro glass cuvette / 96 well flat-bottom plate and distilled water.

Protocol:

I. Sample Extraction:

Isolation of cytoplasmic proteins and mitochondrial proteins from tissues, bacteria, or cells:

1. Take 0.1 g of tissue or 5 million cells, add 1 mL of Extract solution and 10 μL of Reagent I, homogenate on ice with homogenizer.
2. Centrifuge at 600 ×g and 4℃ for 5 minutes.
3. Take the supernatant to another centrifuge tube, centrifuge at 11000 ×g and 4℃ for 10 minutes.
4. The supernatant is a plasma extract that can be used to determine CS leakage from mitochondria.

5. Add 200 μL of Reagent I and 2 μL of Reagent II into precipitate, mix thoroughly to detect the activity of

CS and the detection of protein concentration.

II. Procedure

1. Preheat spectrophotometer for 30 minutes, adjust wavelength to 412 nm, set zero with distilled water.

2. Preheat Reagent III at 25℃(general species) or 37℃(mammals) water bath for 10 minutes（guaranteed no precipitation）.

3. Procedure test

Reagent name (μL)	Test tube (T)	Control tube (C)
Reagent III	172	186
Reagent IV	7	7
Reagent V	7	-
Sample	7	7
Reagent VI	7	-

Add reagents orderly to the micro glass cuvette/96 well flat-bottom plate, record the time when adding Reagent VI, record the absorbance A1 of 10s at 412 nm. Then place the cuvette with reaction solution to 37℃ or 25℃ water bath for 2 min (96-well flat-bottom plate is put into the incubator). Take out and wipe to dry the cuvette, record the absorbance A2 of 412 nm at 130s, test tube and control tube all need detect.

Test tube: ΔA1=A2-A1, Control tube ΔA1'=A2-A1. ΔA=ΔA1-ΔA1'.

III. Calculation

A. Micro quartz cuvette

(1). Protein concentration

Unit definition: One unit of enzyme activity is defined as the amount of enzyme catalyzes the generation of 1 nmol TNB at 37℃ or 25℃every milligram of tissue protein per minute.

$CS(U/mg\ prot)=\Delta A\div(\varepsilon\times d)\times V_{RT}\div(Cpr\times V_{S)}\div T=1050\times\Delta A\div Cpr$

ε: Molar extinction coefficient of TNB, 13.6×10-3mL/(nmol·cm);

V_{RT}: Reaction volume, 0.2 mL;

d: Cuvette diameter(cm), 1 cm;

Vs: Sample volume, 0.007 mL;

T: Reaction time(min), 2 minutes;

Cpr: Protein concentration after precipitation dissolution, mg/mL.

B. 96 well flat-bottom plate (UV plate)

Change the d-1 cm in the above formula to d-0.6 cm for calculation.

Note:

1. Samples and all reagents place on ice, in order to avoiding denaturation and lose activity.

2. The reaction solution of cuvette should place 37℃ or 25℃. Add a certain amount of distilled water to a small beaker, then the small beaker place in water bath at 37℃ or 25℃. Place the cuvette with the solution in the beaker during the reaction.

3. Two people do this experiment at the same time, one person colorimetric, the other person timing to ensure the accuracy of experiment results.

4. It is recommended to use the concentration of sample protein to calculate the enzyme activity. If the fresh weight of sample is used to calculate, the enzyme activity of enzyme solution extracted from cytoplast should be measured. The sum of enzyme activity in supernatant and precipitation is the total enzyme activity.

5. Detect with 96 well flat-bottom plate, prepare working solution of test tube and contrast tube according to the test tube's number. It is not recommended to measure multiple samples at the same time because the enzyme activity is calculated by changing the absorptivity per unit time.

6. Appendix: Calculation formula of sample weight.

A. Micro quartz cuvette

Unit definition: One unit of enzyme activity is defined as the amount of enzyme catalyzes the generation of 1 nmol TNB at 37℃ or 25℃ every gram tissue per minutein the reaction system.

$CS_S(U/mg\ prot)=\Delta A_S\div\varepsilon\div d\times V_{RT}\div(W\times V_S\div V_E)\div T=1061\times\Delta A_S\div W$

$CS_P(U/mg\ prot)=\Delta A_P\div\varepsilon\div d\times V_{RT}\div(W\times V_S\div V_P)\div T=212\times\Delta A_P\div W$

$CS(U/mg\ prot)=CS_S+CS_P=1061\times\Delta AS\div W+212\times\Delta A_S\div W$

ΔA_1: The measured value of supernatant;

ΔA_2: The measured value of precipitation;

ε: Molar extinction coefficient of TNB, 13.6×10^{-3} mL/(nmol·cm);

V_{RT}: Reaction total volume, 0.2 mL；

d: Cuvette diameter(cm), 1 cm;

Vs: Sample volume, 0.007 mL;

$V_{E:}$ Extract solution volume, 1.01 mL;

V_P: Total volume of precipitation, 0.202 mL;

T: Reaction time(min), 2 minutes.

W: Sample weight, g;

B. 96 well flat-bottom plate(UV plate)

Change the d-1 cm in the above formula to d-0.6 cm for calculation.

Experimental instances：

1. Take 0.1g of mouse heart, add 1mL of Reagent I and 10μL of Reagent II, homogenate and grind. Take supernatant and centrifuge, take the supernatant and sediment. Add 200μL of Reagent I and and 2μL of Reagent II to the sediment, test according to the measured steps. Calculate in the supernatant: $\Delta A1=A2-A1=1.4577-0.8015=0.6562$， $\Delta A1'=A2'-A1'=0.7842-0.7331=0.0511$， $\Delta A_S=\Delta A1-\Delta A1'=0.6051$, Calculate in the precipitation: $\Delta A1=A2-A1=0.4166-0.1303=0.2863$， $\Delta A1'=A2'-A1'=0$， $\Delta A_P=\Delta A1-\Delta A1'=0.2863$, calculate the enzyme activity according to sample weight:

$CS（U/g\ weight）=CS_S+CS_P=1061\times\Delta A_S\div W+212\times\Delta A_P\div W=1061\times0.6051\div0.1+212\times0.2863\div0.1=7027.067$ U/g weight。

Recent Product citations:

[1] Ming Song,FangfangChen,YihuiLi,et al. Trimetazidine restores the positive adaptation to exercise

training by mitigating statin-induced skeletal muscle injury. Journal of Cachexia, Sarcopenia and Muscle. November 2017;(IF10.754)

[2] Zhang J, Lv J, Xie J, et al. Nitrogen Source Affects the Composition of Metabolites in Pepper (Capsicum annuum L.) and Regulates the Synthesis of Capsaicinoids through the GOGAT–GS Pathway[J]. Foods, 2020, 9(2): 150.

References:

[1]Agostinho F R, Réus G Z, Stringari R B, et al. Treatment with olanzapine, fluoxetine and olanzapine/fluoxetine alters citrate synthase activity in rat brain[J]. Neuroscience letters, 2011, 487(3): 278-281.

Related products：

NA0843/NA0601 CoenzymeINAD(H) Content Assay Kit
NA0795/NA0554 NAD Kinase (NADK) Assay Kit
NA0818/NA0576 NADH oxidase(NOX) Activity Assay Kit
NA0785/NA0544 NAD Malic Enzyme(NAD-ME) Assay Kit