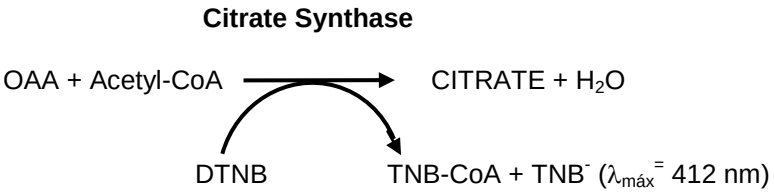
Citrate Synthase Enzyme Activity Assay

Catalog Number: CSE1
Store at -20°C.
FOR RESEARCH USE ONLY



The enzyme citrate synthase, located at the mitochondria matrix, stands as a rate-limiting enzyme in the first step of the Citric Acid Cycle (or Krebs Cycle). Citrate synthase catalyzes the condensation reaction of the acetate residue from acetyl coenzyme A and oxaloacetate to form citrate. Oxaloacetate will be regenerated after the completion of one round of the Krebs Cycle. The enzyme activity is used to assess the oxidative capacity of the mitochondria as well as mitochondria integrity. The reaction capacity of the citrate synthase can be followed by measuring the formation of the -SH group released from CoA-SH by use of the reactive Ellman reagent (5,5`-dithiobis [2-nitrobenzoic], DTNB) and monitoring the absorbance at 412 nm.

Contents:

- 96 well plate.
- Extraction Buffer.
- Reaction buffer.
- DTNB mix.
- Positive control, Citrate Synthase enzyme (5mU/µl) - Do not freeze.
- Acetyl-CoA
- Oxaloacetate mix
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Not Included:

Mitochondrial isolation buffer

Sample preparation

- Hard Tissue: Homogenize tissue in a knife homogenizer (Polytron, Turrax or quickly sonication) at a ratio of 4 ml of extraction buffer per gram of tissue and centrifuge at 1,000g for 15 min at 4 ° C. Collect the supernatant, measure proteins (optimal concentration of around 0.5 mg / ml).
- Cells: Sonicated or homogenized in a Potter-Elvehjem. Centrifuge at 1,000g for 10 min at 4 ° C, collecting the supernatant, measure protein (optimal concentration of around 0.5 mg / ml).
- Mitochondria rich fraction: Since 92% citrate synthase activity is located in the mitochondria and acetyl-CoA cannot penetrate therein, the preparations may be made by separating the mitochondria (to increase yield) and breaking up the same. Homogenize sample with mitochondria homogenization buffer (250 mM sucrose, 2 mM ethylenediaminetetraacetic acid (EDTA), and 25 mM Tris-HCl, pH 7.4) plus protease inhibitor. Centrifuge at 800g for 10 minutes 4ºC. Careful collect the supernatant (discard pellet) and centrifuge at 15.000-20.000 x g for 20 minutes at 4ºC. Wash the pellet by adding PBS and centrifuging 15.000-20.000 x g for 5 minutes. Resuspend pellet with extraction buffer.

Reagents preparation

- Thaw all reagents.
- Add 985µl of reaction buffer in DTNB mix tube (stable for one day on ice).

Assay (415 nm a 37°C)

- 20µl sample (total protein rage 5-20 µg/ml)
- 10µl reaction buffer
- 5 µl of acetil-CoA
- 15µl sample buffer
- Add 100 µl to plate blank
- Mix for 10 seconds and incubate at 37°C for 1 minute.
- Add 50 µl of oxaloacetate mix and quickly mix for 5 seconds and read during 3-8 minutes.

Reaction blanks- Add extraction buffer instead sample in reaction buffer and acetyl-CoA and oxaloacetate mix sample (in addition to the ELISA plate blanks) this activity should be deducted from the sample values.

Calculation:

ε TNB / 412 nm = 13.6 mM-1.cm-1 but at 415 nm (Elisa readers of filter) the ε_{TNB} = 7.351.Therefore, 7,351 (µmol / ml) -1.cm-1, in 100 µl we have: 73.51 $\mu mol^{-1}.cm^{-1}$ and the optical path of 100 µl in the microplate 0.221 cm. Therefore, we have: 16.2457 μmol^{-1} or 0.0162457^{-1} nmol. Thus, applying the conversion factor absorbance units per minute for nmols per minute, the calculation is:

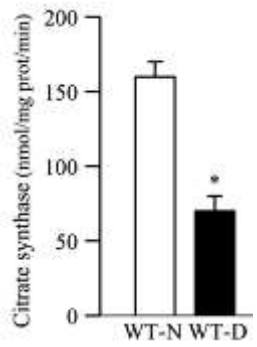
Enzyme activity in nmols/min/mg protein =

$$\frac{\text{(Units of absorbance/min)}}{0{,}0162457 \times \text{mg of protein in the assay}}$$

1 mU of Citrate Synthase = 1 nmol/min a 37°C

References:

[1] PA Srere.Citrate synthase.Methods Enzymol., 13:3-11, 1969.
[2] D Shepherd & PB Garland.Citrate synthase from rat liver. Methods Enzymol., 13:11-16, 1969.
[3] E Bogin & A Wallace.Citrate synthase from lemon fruit. Methods Enzymol., 13:19-22, 1969.
[4] J. Santos et al. Free Radical Biology and Medicine 51: 1849-1860, 2011



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