Citrate Synthase
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Summary

Citrate synthase catalyzes the condensation of acetyl CoA and oxaloacetate to citrate and CoA. This protocol describes an assay which uses DTNB, a chemical which reacts with sulfhydryl groups such as that in the CoA generated in this enzymatic reaction.

Solutions Required

1. 40 mM Tris HCl buffer (FW 157.6)
   adjust to a pH of 8.1 with 20% KOH
   can be stored in stock

2. 5.0 mM Acetyl CoA (sodium salt FW 809.6)
   can be prepared in stock solution and stored at -20°C

3. 100 mM oxaloacetate (FW 132.1)
   can be prepared in stock solution and stored at -20°C
   prepare a 200 mM oxaloacetic acid solution and promptly neutralize with an equal volume of 400 mM $KHCO_3$

4. 10.0 mg DTNB in 10 mL 100% ethanol (FW)
   must be prepared fresh

5. 100 mM KCl

Preparation of Cell Extract

Follow general protocol **Preparation of Cell Extract**.

1. Centrifuge sufficient cells so that the volume diluted down to 2 mL would give an optical density of 40-50. For example, for a broth of OD=1, use 100 mL. For a broth of OD=10, use 10 mL.
2. After first pelletization of cells, resuspend in 5-15 mL of 4°C tris buffer.
3. After second pelletization of cells, resuspend in 2 mL of 4°C tris buffer, and break with French Press.

Spectrophotometer

Turn on the ultraviolet and visible bulb on the spectrophotometer (Beckman DU50) and wait 20 minutes for warm-up. Select the kinetics-time window on the instrument. Use a run-time of 60 s, a temperature of 37°C, a wavelength of 412 nm and 2 autosamplers.

Procedure

1. For each assay, prepare the two cocktails shown in the following table into two separate UV-translucent cuvettes, and keep them on ice.

Solution	Volume (µL) added to: Control	Experimental
DI H_2O	100	0
Tris	500	500
DTNB	100	100
Acetyl CoA	100	100
Oxaloacetate	0	100
KCl	100	100

2. Directly from the ice when ready to commence the assay, place the two cuvettes (each containing 900 µL†) into the spectrophotometer holder (position #1 for control, position #2 for experimental).
3. Depress "read samples" on the monitor.
4. Wait 5 minutes to allow the temperature of the solutions in the cuvettes to equilibrate.
5. Blank. Depress "read sample" on the monitor.
6. Simultaneously add 100 µL† of the cell extract to the cuvettes.
7. To mix solutions, immediately and simultaneously aspirate and dispense the contents of the cuvettes with a pipettor.
8. Promptly depress "start" on the monitor.
9. Record the rates for the two (control and experimental) cuvettes.

† Volume of cell extract may be adjusted so that change in absorbance is between about 0.05 and 0.7 AU in one minute. Any change in cell extract should be accompanied by a change in the DI water in the cocktails. (For example, if 50 µL of cell extract were used, then 50 µL of DI water would be used in the experimental.)

Calculation of Activity

One unit (U) of pyruvate carboxylase activity is defined as the amount of enzyme required to produce 1.0 µmole of oxaloacetate in one minute.

1. $dA/dt\ (min^{-1}) = [Rate]_{experimental} - [Rate]_{control} = dA/dt$

2. $$\text{Activity} = \frac{1000 \times TV \times dA/dt}{\varepsilon \times V \times CF}$$

Activity:	Volumetric Activity (U/L)
TV:	Total volume in cuvette (1000 µL)
V:	Volume of cell extract used (100 µL)
ε:	Molar extinction coefficient for reduced DTNB (13.6 L/mmol for a path length of 1.0 cm)
D:	Concentration factor for cell extract (For example, if a 100 mL sample is concentrated to a 2 mL volume for the French Press, then CF=50)

3. $$\text{Specific Activity} = \frac{Activity}{Protein\ Concentration}$$

Activity:	Volumetric Activity, as calculated in #2 above (U/L)
Protein Concentration:	Protein concentration, as calculated in protocol **Total Protein Concentration** (mg/L)
Specific Activity:	(U/mg protein)

Reference

P. A. Srere, H. Brazil, L. Gonen. 1963. The citrate condensing enzyme of pigeon breast muscle and moth flight muscle. *Acta Chemica Scandinavica*, 17, S129-S134.