Isocitrate Lyase
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Summary

Isocitrate lyase catalyzes the hydrolysis of isocitrate into glyoxylate and succinate. This protocol describes an assay which relies on a specific reaction of the product glyoxylate.

Solutions Required

1. 250 mM potassium phosphate buffer pH = 7.0.
   prepared by mixing 36 mL of 250 mM KH_2PO_4 and 45 mL of 250 mM K_2HPO_4.

2. 0.1 M $MgCl_2$
   can be prepared in stock solution and stored at room temperature.

3. 0.1 M cysteine HCl
   must be prepared fresh

4. 0.1 M phenylhydrazine HCl
   must be prepared fresh

5. 0.1M isocitrate
   can be prepared in stock solution and stored at 4°C for a couple weeks.

Preparation of Cell Extract

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

1. Centrifuge sufficient cells so that the volume diluted down to 5 mL would give an optical density of 20-30. 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 potassium phosphate buffer.
3. After second pelletization of cells, resuspend in 5 mL of potassium phosphate buffer, and break with French Press.

Spectrophotometer

Turn on the ultraviolet bulb on the spectrophotometer (Beckman DU50) and wait 30 minutes for warm-up. Select the kinetics-time window on the instrument. Load the method "A:/icl". This method has a run-time of 60 s, a temperature of 37°C (or another appropriate fermentation temperature), a wavelength of 324 nm and uses 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.

	Volume (µL) added to:	
Solution	Control	Experimental
DI H_2O	330	250
Potassium Phosphate	400	400
$MgCl_2$	60	60
Phenylhydrazine	40	40
Cysteine	120	120
Isocitrate	0	80

2. Directly from the ice when ready to commence the assay, place the two cuvettes (each containing 950 µL†) into the spectrophotometer holder (position #1 for control, position #2 for experimental).
3. Wait 10 minutes to allow the temperature of the solutions in the cuvettes to equilibrate.
4. "Blank" and then depress "Read Samples" on the monitor.
5. Simultaneously add 50 µL† of the cell extract to the cuvettes.
6. To mix solutions, immediately and simultaneously aspirate and dispense the contents of the cuvettes with a pipettor. Mix the solutions in this way ten times. (Count!)
7. Promptly depress “start” on the monitor.
8. Record the rates for the two (control and experimental) cuvettes.

† Dilution of the cell extract may be adjusted so that change in absorbance is between about 0.05 and 0.7 AU in one minute. This dilution should be accomplished externally in a microcentrifuge tube (for example, by adding 50 µL of cell extract to 950 µL DI water to achieve a dilution of 20). The volume of 50 µL should always be used in the enzyme assay mixture.

Calculation of Activity

One unit (U) of isocitrate lyase activity is defined as the amount of enzyme required to produce 1.0 μmole of glyoxylate in one minute.

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

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

Activity:	Volumetric Activity (U/L)
TV:	Total volume in cuvette (1000 μL)
D:	Dilution of the cell extract. (For example, if 50 μL of cell extract were add to 950 μL DI water prior to using a volume of cell extract in the assay, then D=20)
V:	Volume of cell extract used (50 μL)
ε:	Molar extinction coefficient for NADH (6.22 L/mmol for a path length of 1.0 cm)
CF:	Concentration Factor of cell extract (For example, if a 100 mL sample is concentrated to a 2 mL volume for the French Press, then CF=50)

3. $$\text{Specific Activity} = \frac{Activity}{Protein\ Concentration} 1$$

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

G. H. Dixon, H. L. Kornberg (1959) Assay Methods for Key Enzymes of the Glyoxylate Cycle. Proceedings of the Biochemical Society, 3P.