Pyruvate Oxidase
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Summary

Pyruvate oxidase catalyzes the oxidation of pyruvate to acetate and carbon dioxide. This protocol describes the measurement of pyruvate oxidase based on the loss of absorbance of ferricyanide as it oxidizes enzyme-bound flavin.

Solutions Required

1. 250 mM potassium phosphate buffer pH = 6.0.
   prepared by mixing xxx mL of 250 mM KH_2PO_4 and xxx mL of 250 mM K_2HPO_4.

2. 100 mM $MgCl_2$

3. 1 mM sodium thiamine pyrophosphate (cocarboxylase, Sigma C8754)
   must be prepared fresh

4. 80 mM $K_3Fe(CN)_6$ (ferricyanide)
   must be prepared fresh

5. 2.0 M pyruvate
   must be prepared fresh

Preparation of Cell Extract

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

1. After first pelletization of cells, resuspend at 4°C in potassium phosphate buffer.
2. After second pelletization of cells, resuspend at 4°C in potassium phosphate buffer.

Spectrophotometer

Turn on the ultraviolet bulb on the spectrophotometer (Beckman DU50) and wait 10 minutes for warm-up. Select the kinetics-time window on the instrument. Load the method "A:/pox". This method has a run-time of 60 s, a temperature of 25°C, a wavelength of 450 nm and use 2 autosamplers.

Procedure

1. For each assay, prepare the two cocktails shown in the following table into two separate cuvettes. Keep at 25°C.

Solution	Volume (µL) added to: Control	Experimental
phosphate buffer	400	400
DI H_2O	200	100
$MgCl_2$	100	100
Thiamine pyrophosphate	100	100
Pyruvate	0	100

2. Into each cuvette add 100 µL of cell extract. Mix cuvettes.
3. Wait 20 minutes. Place cuvettes into spectrophotometer.
4. "Blank" and then depress "Read Samples" on the monitor.
5. Simultaneously into each cuvette add 100 mM of potassium ferricyanide.
6. To mix solutions, immediately and simultaneously aspirate and dispense the contents of the cuvettes with a pipettor.
7. Promptly depress "start" on the monitor.
8. 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 150 µL of DI water would be used in the experimental.)

Calculation of Activity

One unit (U) of pyruvate oxidase activity is defined as the amount of enzyme required to consume 1.0 µmole of pyruvate in one minute. Note that two equivalents of ferricyanide are reduced per equivalent of pyruvate decarboxylated.

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

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

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 ferricyanide (0.218 L/mmol for a path length of 1.0 cm)

CF: Dilution of cell extract (For example, if a 100 mL sample is concentrated to a 5 mL volume for the French Press, then CF=20)

2: 2 equivalents $Fe(CN)_6^{3-}$ per equivalent pyruvate

3. 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)

References

R. Blake II, L. P. Hager, R. G. Gennis. 1978. Activiation of pyruvate oxidase by monomeric and micellar amphiphiles. *Journal of Biological Chemistry*, 253(6):1963-1971.

Y.-Y. Chang, J. E. Cronan, Jr. 1982. Mapping nonselective genes of *Escherichia coli* by using transposon Tn10: Location of a gene affecting pyruvate oxidase. *Journal of Bacteriology*, 151:1279-1289.