Pyruvate Kinase
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Summary

Pyruvate kinase dephosphorylates phosphoenolpyruvate (PEP) into pyruvate using ADP as the phosphate group acceptor, according to the reaction

$$PEP + ADP \rightarrow Pyruvate + ATP$$

This protocol describes an indirect assay to determine the activity of pyruvate kinase. Pyruvate formed from PEP by pyruvate kinase is measured by the formation of NAD^+ in presence of lactate dehydrogenase.

Solutions Required

1. 50 mM HEPES (N-2-hydroxyethylpiperazine-N'-2-ethane sulfonic acid) pH 7.5
   must not be more than 1 week old

2. 100 mM $MgCl_2$
   Stock solution could be used

3. 500 mM KCl
   Stock solution could be used

4. 40 mM ADP
   Must be prepared fresh

5. 100 mM PEP
   Must be prepared fresh

6. 10 mM NADH
   Must be prepared fresh

7. 22 units of lactate dehydrogenase

Preparation of Cell Extract

Follow general protocol described in **Preparation of Cell Extract**. The cell extract should be suspended in HEPES buffer after pelletization.

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:/nadh" ".

These methods each have a run-time of 120 s, a temperature of 25°C, a wavelength of 340 nm and use 2 autosamplers.

Procedure

1. For each assay, prepare the two cocktails shown in the following table into two separate quartz cuvettes. Keep them on ice.

	Volume (µL) added to:	
Solution	Control	Experimental
HEPES	200	200
DI H_2O	428	328
$MgCl_2$	100	100
KCl	100	100
ADP	50	50
PEP	0	100
NADH	50	50
lactate dehydrogenase	22	22

2. Directly from the ice, 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. Depress "Blank" and then depress "Read Samples" on the monitor.
5. Simultaneously add 50 µL† of the cell extract to the cuvettes.
6. Mix the solutions using a pipettor and promptly depress “start” on the monitor. Mix the solutions in this way ten times. (Count!)
7. 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 pyruvate kinase activity is defined as the amount of enzyme required to produce 1.0 µmole of pyruvate from PEP 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. $$\text{Specific Activity} = \frac{Activity}{Protein\ Concentration}$$

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

M. Malcovati and G. Valentini. 1982. AMP- and Fructose 1,6,-Biphosphate-activated pyruvate kinases from *Escherichia coli*. *Methods in Enzymology,* 90:170-179.