Glucokinase
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Summary

Glucokinase phosphorylates glucose into glucose 6-phosphate using ATP as the phosphate group donor, according to the reaction

glucose + ATP → glucose 6-phosphate + ADP

This protocol describes an indirect assay to determine the activity of glucokinase. Glucose 6-phosphate formed by glucokinase is measured by the formation of NADPH in presence of glucose 6-phosphate dehydrogenase.

Solutions Required

1. 100 mM tris HCl pH 7.4
   Stock solution could be used

2. 100 mM $MgSO_4 \cdot 7H_2O$
   Stock solution could be used

3. 10 mM glucose
   Must be prepared fresh

4. 20 mM ATP
   Must be prepared fresh

5. 10 mM NADP
   Must be prepared fresh

6. a solution containing 10 U of glucose 6-phosphate dehydrogenase per 50 µL in tris HCl pH 7.4 buffer. This solution is prepared by mixing 40 µL of a 5000 U/mL solution (Sigma G8529) with 960 µL tris buffer.

Preparation of Cell Extract

Follow general protocol described in **Preparation of Cell Extract**. The cell extract should be suspended in tris 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 37°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
tris	500	500
DI H_2O	200	100
$MgSO_4$	100	100
glucose	0	100
ATP	50	50
NADP	50	50
G6P dehydrogenase	50	50

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 5-8 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 glucokinase activity is defined as the amount of enzyme required to produce 1.0 μmole of glucose 6-phosphate from glucose 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 NADPH (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

A. M. Pakoskey, E. C. Lesher, D. B. M. Scott. 1965. Hexokinase of *Escherichia coli*. Assay of enzyme activity and adaptation to growth in various media. *J. Gen. Microbiol.* 38:73-80.