Megazyme

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ACETIC ACID GK
(ADP-Glucokinase Format)

ASSAY PROCEDURE
FOR AUTO-ANALYSER
APPLICATIONS

K-ACETGK 04/20

(500 Assays per Kit)

PRINCIPLE:

Acetic acid is phosphorylated to form acetyl-phosphate in the reaction catalysed by acetate kinase (AK) (1).

$$\text{(1)}\quad \text{Acetic acid} + \text{ATP} \xrightarrow{\text{(AK)}} \text{acetyl-phosphate} + \text{ADP}$$

The rapid conversion of the acetyl-phosphate product into acetyl-CoA and inorganic phosphate is catalysed by the action of phosphotransacetylase (PTA) in the presence of coenzyme A (CoA) (2).

$$\text{(2)}\quad \text{Acetyl-phosphate} + \text{CoA} \xrightarrow{\text{(PTA)}} \text{acetyl-CoA} + P_i$$

D-Glucose is phosphorylated by the enzyme ADP-glucokinase (ADP-GK) and adenosine-5'-diphosphate (ADP) to glucose-6-phosphate (G-6-P) with the simultaneous formation of adenosine-5'-monophosphate (AMP) (3).

$$\text{(3)}\quad \text{ADP} + \text{D-glucose} \xrightarrow{\text{(ADP-GK)}} \text{glucose-6-phosphate} + \text{AMP}$$

In the presence of the enzyme glucose-6-phosphate dehydrogenase (G6P-DH), G-6-P is oxidised by nicotinamide-adenine dinucleotide (NAD^+) to gluconate-6-phosphate with the formation of reduced nicotinamide-adenine dinucleotide (NADH) (4).

$$\text{(4)}\quad \text{Glucose-6-phosphate} + NAD^+ \xrightarrow{\text{(G6P-DH)}} \text{6-phosphogluconate} + \text{NADH} + H^+$$

KITS:

Kits suitable for performing 500 assays in auto-analyser format are available from Megazyme. The kits contain the full assay method plus:

Bottle 1: Buffer (11 mL, pH 7.4).
Stable for > 2 years at 4°C.

Bottle 2: NAD^+, ATP, D-glucose, CoA and PVP.
Freeze dried powder.
Stable for > 5 years below -10°C.

Bottle 3: Acetate kinase, phosphotransacetylase, ADP-glucokinase plus glucose-6-phosphate dehydrogenase suspension, 2.6 mL.
Stable for > 2 years at 4°C.

Bottle 4: Acetic Acid Standard (2 mL)
(1.8 g/L). Ready to use.
Stable for > 2 years; store sealed at 4°C.

PREPARATION OF REAGENT SOLUTIONS/SUSPENSIONS:

1,3 & 4. Use the contents of bottles 1, 3 and 4 as supplied. Stable for > 2 years; store sealed at 4°C.

2. Dissolve the contents of bottle 2 in 11 mL of distilled water. **This is reagent R2 and is stable for > 1 week at 4°C or > 2 years below -10°C** (this reagent is stable when subjected to freeze/thaw cycles, however to avoid repetitive freeze/thaw cycles, divide into appropriately sized aliquots and store in polypropylene tubes).

REAGENT PREPARATION:

Preparation of R1:

Component	Volume
distilled water bottle 1 (buffer) suspension 3 (AK/PTA/ADP-GK/G6P-DH)	87.5 mL 10 mL 2.5 mL
Total volume	100 mL

Preparation of R2:

Component	Volume
bottle 2 (NAD^+/ATP/D-glucose/CoA/PVP)	11 mL (after adding 11 mL of distilled water)
Total volume	11 mL

EXAMPLE METHOD:

R1:	0.200 mL
Sample:	~ 0.003 mL
R2:	0.020 mL
Reaction time:	~ 8 min at 25°C or ~ 5 min at 37°C
Wavelength:	340 nm
Prepared reagent stability:	
R1:	> 30 days 4°C/> 2 years below -10°C
R2:	> 7 days 4°C/> 2 years below -10°C
Calculation:	endpoint
Reaction direction:	increase
Linearity:	up to 1.8 g/L of acetic acid

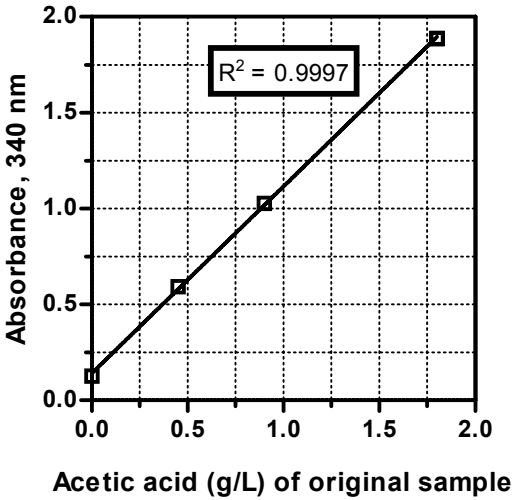

Figure 1. Calibration curve demonstrating the linearity of **K-ACETGK**. The reactions used to generate this calibration curve were performed at 37°C for 5 min using a Chemwell-T auto-analyser.

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