

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).



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).



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).



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).



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 (1 l 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	87.5 mL
bottle 1 (buffer)	10 mL
suspension 3 (AK/PTA/ADP-GK/G6P-DH)	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

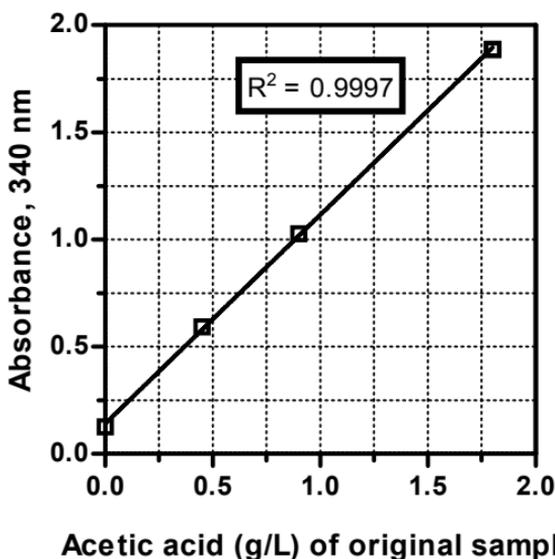


Figure 1. Calibration curve demonstrating the linearity of **K-ACETGK**. The reactions used to generate this calibration curve were performed at 37°C for 5 min using a Chemwell-T auto-analyser.



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