Megazyme

ACETIC ACID (ACETATE KINASE FORMAT)

ASSAY PROCEDURE FOR AUTO-ANALYSER APPLICATIONS

K-ACETAK 06/13

INTRODUCTION:

The most widely used method for enzymatic quantification of acetic acid is that employing acetyl-coenzyme A synthetase (ACS). However, this method is based on the use of an indicator reaction catalysed by L-malate dehydrogenase that is in permanent equilibrium, and therefore a non-stoichiometric increase in absorbance is observed from the acetate present in the sample. Thus, a slightly non-linear response to increasing acetate concentration is observed upon calibration, resulting in poor R^2 values. In addition (depending on the supplier) reagents prepared for auto-analyser applications can have very limited on-machine stability, due to rapidly increasing blank absorbance values. To overcome these issues, Megazyme developed this alternative acetic acid kit, based on the enzyme acetate kinase (AK; see equations 1-3 below), especially for the auto-analyser user. This reagent has improved on-machine stability, gives excellent linear calibration curves, and results in a stoichiometric change in absorbance due to the acetic acid present in the sample.

PRINCIPLE:

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

(2) ADP + PEP $\xrightarrow{\text{(pyruvate kinase; PK)}}$ ATP + pyruvate

(3) Pyruvate + NADH + H^+ $\xrightarrow{\text{(D-lactate dehydrogenase; D-LDH)}}$ D-lactic acid + NAD^+

KITS:

Kits suitable for performing a minimum of 550 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) plus sodium azide (0.02% w/v) as a preservative. Stable for > 2 years at 4°C.

Bottle 2: NADH plus ATP, PEP and PVP.
Stable for > 2 years at either 4°C or -20°C.

Bottle 3: Acetate kinase plus pyruvate kinase and D-lactate dehydrogenase suspension, 4.1 mL.
Stable for > 2 years at 4°C.

PREPARATION OF REAGENT SOLUTIONS/SUSPENSIONS:

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

2. Dissolve the contents of bottle 2 in 20 mL of distilled water. **Stable for > 1 year at 4°C** or stable for > 2 years at -20°C (to avoid repetitive freeze / thaw cycles, divide into appropriately sized aliquots and store in polypropylene tubes).

REAGENT PREPARATION:

Preparation of R1:

Component	Optical path-length (mm) of analyser				
	6	7	8	9	10
Bottle 2 (after resuspension in 20 mL of water)	3.9 mL	3.3 mL	2.8 mL	2.2 mL	2.2 mL
distilled water	36.1 mL	36.7 mL	37.2 mL	37.8 mL	37.8 mL
Total Volume	40 mL				

Preparation of R2:

Component	Volume
Bottle 1 Bottle 3 (AK/PK/L-LDH)	2.5 mL 1 mL
Total volume	3.5 mL

EXAMPLE METHOD:

R1: 0.290 mL
Sample: ~ 0.005 mL
R2: 0.020 mL

Reaction time: ~ 10 min at either 20-25°C or 37°C
Wavelength: 340 nm
Prepared reagent stability: > 7 days when refrigerated
Calculation: endpoint
Reaction direction: decrease
Linearity: up to 30 µg/mL of acetic acid in the final reaction solution (equivalent to 0-1.8 g/L for the method described above, employing 0.005 mL of sample and a path-length of 4.6 mm).

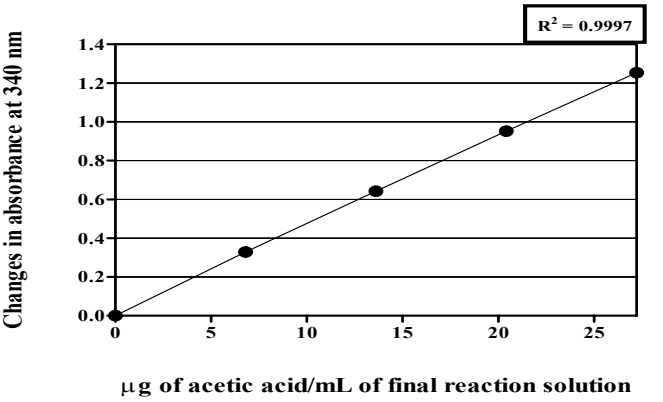

Figure 1. Calibration curve demonstrating the linearity of K-ACETAK. The reactions used to generate this calibration curve were performed at 25°C for 10 min, using a 4.6 mm path-length cuvette.

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