

# Megazyme

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## ACETIC ACID (ACETATE KINASE FORMAT)

### ASSAY PROCEDURE FOR AUTO-ANALYSER APPLICATIONS

K-ACETAK 06/07

(170.5 mL of reagent [R1 + R2] per kit;  
equivalent to 550 reactions of 0.31 mL)

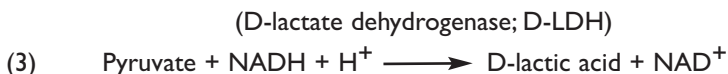
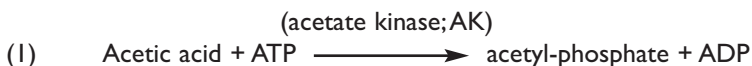
*This Data Booklet will soon be available at*  
**[www.megazyme.com](http://www.megazyme.com)** in the following languages  
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## INTRODUCTION:

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) reagent 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:



## KITS:

Kits suitable for the preparation of 170.5 mL of reagent (equivalent to 550 reactions of 0.31 mL) are available from Megazyme.

The kits contain the full assay method plus:

**Bottle 1:** Tris/HCl buffer (20 mL, 1 M, pH 7.4) plus magnesium chloride (30 mM) and sodium azide (0.02 % w/v) as a preservative.

Stable for > 2 years at 4°C.

**Bottle 2:** Tablets (30), containing NADH plus ATP, PEP and PVP. Stable for > 2 years at either 4°C or -20°C.

**Bottle 3:** Acetate kinase (15,500 U/mL) plus pyruvate kinase (1,450 U/mL) and D-lactate dehydrogenase (1000 U/mL) suspension, 4.1 mL.

Stable for > 2 years at 4°C.

## REAGENT PREPARATION:

### Preparation of R1:

Component	Optical path-length (mm) of analyser				
	6 e.g. Alcyon	7 e.g. KoneLab	8 e.g. plate reader	9	10 e.g. Smartchem
Bottle 1 (buffer)	4 mL	4 mL	4 mL	4 mL	4 mL
Bottle 2 (tablets)	7	6	5	4	4
H <sub>2</sub> O	36 mL	36 mL	36 mL	36 mL	36 mL
Total volume	40 mL				

(**NB:** the tablets will fully dissolve after a few min with gentle shaking or swirling. It is important to add the water component last, i.e. to the tablet/buffer mixture).

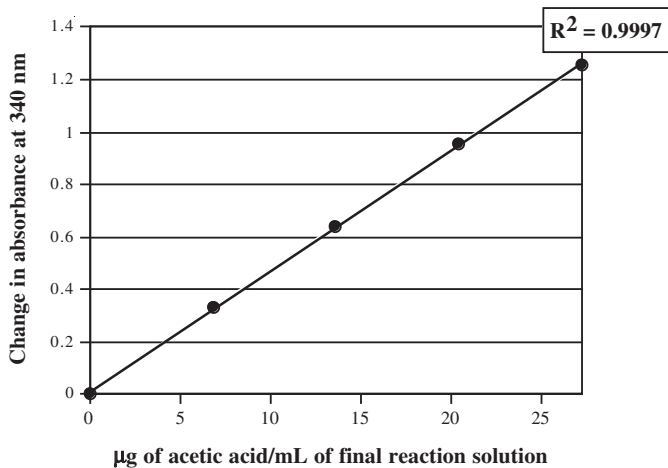
### Preparation of R2:

Component	Volume
Bottle 3 (AK/PK/L-LDH)	1.00 mL (swirl to mix before use)
H <sub>2</sub> O	1.75 mL
Total volume	2.75 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 decrease  
**Reaction direction:** decrease  
**Linearity:** up to 30 µg/mL of acetic acid in 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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