

Megazyme

ACETIC ACID (Acetyl-CoA Synthetase Format)

ASSAY PROCEDURE FOR AUTO-ANALYSER APPLICATIONS

K-ACETAF 12/07

(141.6 mL of reagent [R1 + R2] per kit;
equivalent to 456 reactions of 0.31 mL)

This Data Booklet will soon be available at
www.megazyme.com in the following languages
French-German-Italian-Spanish-Portugese

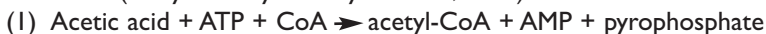


INTRODUCTION:

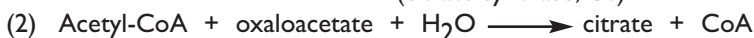
The most widely used method for enzymatic quantification of acetic acid is that based on the use of acetyl-coenzyme A synthetase (ACS), according to equations 1 – 3 below. For auto-analyser applications it becomes necessary to prepare a “master mix” reagent (RI), containing all components of the assay except the reaction initiation enzyme, ACS. However, reagent RI when prepared from some kits has very limited on-machine stability, owing to a rapidly increasing absorbance value. To overcome this issue, Megazyme developed this kit (K-ACETAF), that doesn't exhibit this instability phenomenon. Additionally, the anti-inhibitory compound polyvinylpyrrolidone (PVP) has also been incorporated into the assay to prevent inhibition caused by certain tannins found in grape juice and wine.

PRINCIPLE:

(acetyl-coenzyme A synthetase; ACS)



(citrate synthase; CS)



(L-malate dehydrogenase; L-MDH)



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: TEA buffer (30 mL, 0.8 M, pH 8.4) plus L-malic acid (60 mM), magnesium chloride (20 mM), PVP (2 mg/mL) and sodium azide (0.02 % w/v) as a preservative. Stable for > 2 years at 4°C.

Bottle 2: (x2) NAD⁺ (134 mg) plus ATP (137 mg) and CoA (9.8 mg). Freeze dried powder. Stable for > 5 years at -20°C.

Bottle 3: L-Malate dehydrogenase (1,250 U/mL) plus citrate synthase (180 U/mL) suspension, 2.2 mL. Stable for > 2 years at 4°C.

Bottle 4: Acetyl-coenzyme A synthetase suspension (1.1 mL, 90 U/mL). Stable for > 2 years at 4°C.

REAGENT PREPARATION:

Preparation of R1:

Component	Volume
Bottle 1 (buffer)	5.50 mL
Bottle 2 (NAD ⁺ /ATP/CoA)	2.20 mL (after adding 5.50 mL of H ₂ O to bottle 2)
Bottle 3 (L-MDH/CS)	0.44 mL (swirl to mix before use)
H ₂ O	18.35 mL
Total volume	26.49 mL

Preparation of R2:

Component	Volume
Bottle 4 (ACS)	0.22 mL (swirl to mix before use)
H ₂ O	1.60 mL
Total volume	1.82 mL

EXAMPLE METHOD:

R1: 0.290 mL

Sample: ~ 0.005 mL

R2: 0.020 mL

Reaction time: 15 min at either 20-25°C or 37°C

Wavelength: 340 nm

Prepared reagent stability: > 3 days when refrigerated

Calculation: endpoint

Reaction direction: increase

Linearity: up to 30 µg/mL of acetic acid in final reaction solution

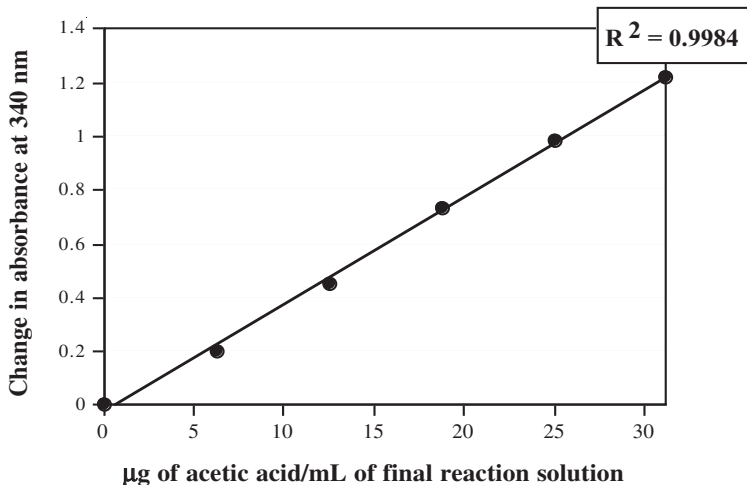


Figure 1. Calibration curve demonstrating the linearity of K-ACETAF. The reactions used to generate this calibration curve were performed at 25°C for 15 min, using a 4.6 mm path-length cuvette.



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