Megazyme

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ACETIC ACID (Acetyl-CoA Synthetase Format)

ASSAY PROCEDURE FOR AUTO-ANALYSER APPLICATIONS

K-ACETAF 04/20

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

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 (R1), containing all components of the assay except the reaction initiation enzyme, ACS. However, reagent R1 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 polyvinylpyrollidone (PVP) has also been incorporated into the assay to prevent inhibition caused by certain tannins found in grape juice and wine.

PRINCIPLE:

(1) Acetic acid + ATP + CoA $\xrightarrow{\text{(acetyl-coenzyme A synthetase; ACS)}}$ acetyl-CoA + AMP + pyrophosphate

(2) Acetyl-CoA + oxaloacetate + H_2O $\xrightarrow{\text{(citrate synthase; CS)}}$ citrate + CoA

(3) L-Malate + NAD^+ $\xrightarrow{\text{(L-malate dehydrogenase; L-MDH)}}$ oxalacetate + NADH + H^+

KITS:

Kits suitable for the preparation of 141.6 mL of reagent (equivalent to 456 reactions of 0.31 mL) are available from Megazyme.

The kits contain the full assay method plus:

Bottle 1: Buffer (30 mL, pH 8.4) plus L-malic acid, PVP and sodium azide (0.02% w/v) as a preservative.
Stable for > 2 years at 4°C.

Bottle 2: (x2) NAD^+ plus ATP and CoA.
Freeze dried powder.
Stable for > 5 years below -10°C.

Bottle 3: L-Malate dehydrogenase plus citrate synthase suspension, 2.2 mL.
Stable for > 2 years at 4°C.

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

Bottle 5: Acetic Acid Standard (2 mL)
(1.8 g/L). Ready to use.
Stable for > 2 years; store sealed 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_2O to bottle 2)
bottle 3 (L-MDH/CS)	0.44 mL (swirl to mix before use)
H_2O	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_2O	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

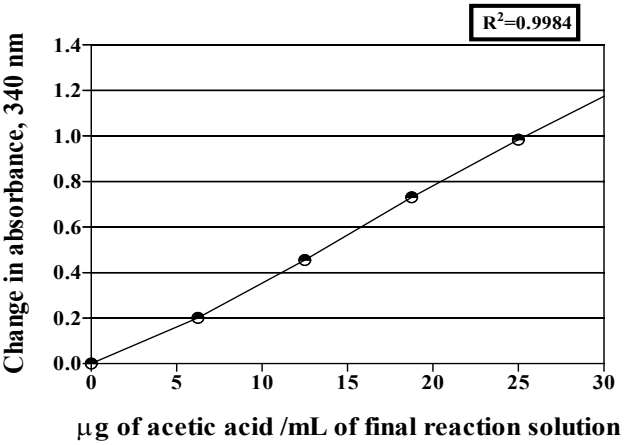

Figure 1. Calibration curve demonstrating the linearity of **K-ACETAF**. The reactions used to generate this calibration curve were performed at 25°C for 15 min, using a 4.6 mm path-length cuvette.

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