ACETIC ACID AUTO

125 ml

INTENDED USE

Reagent for enzymatic determination of acetic acid in foodstuff and other sample material.

PRINCIPLE OF THE METHOD

Acetic acid (acetate) is converted into acetyl-coenzyme A by the enzyme Acetyl-CoA-synthetase (ACS). Acetyl-coenzyme A reacts with oxaloacetate in the presence of Citrate Synthase (CS), to give citrate. Oxaloacetate necessary for the reaction is produced by malic acid and NAD^+ in the presence of enzyme Malate Dehydrogenase (MDH).
Absorbance increase due to NADH formation, measured at 340 nm, is proportional to the amount of acetic acid present in the sample.

KIT COMPONENTS

The components of the kit are stable until expiration date on the label.
Keep away from direct light sources.

ACET AUTO R1A: 1 x 50 ml (liquid) blue cap
Composition: Buffer pH 8.5, $MgCl_2$ > 1 mM, preservatives.

ACET AUTO R1B: 1 x 50 ml (liquid) white cap
Composition: Buffer pH 5.0, ATP > 3 mM, NAD^+ > 3 mM, CoA > 0.05 mM, preservatives.

ACET AUTO R2: 1 x 25 ml (liquid) red cap
Composition: Buffer pH 7.6, ACS > 100 U/l, CS > 500 U/l, MDH > 500 U/l, preservatives.

Store all components at 2-8°C.

In vitro use only.

MATERIALS REQUIRED BUT NOT SUPPLIED

Current laboratory instrumentation. Spectrophotometer UV/VIS with thermostatic cuvette holder. Automatic micropipettes. Glass or high quality polystyrene cuvettes.
Standard solution.
Multiparametric Standard (code SQPE053234) is available on request. Please contact customer service for further information.

REAGENT PREPARATION

Procedure 1:
Reagent R1: mix 1 part of reagent R1A with 1 part of reagent R1B.
It is suggested to prepare strictly the amount needed for the analysis, and any residue has to be stored at 2-8°C away from direct light sources.
Reagent R2 is ready to use.

Analytical performances of reagent R1 begin to fall off 48 hours after the preparation.

Procedure 2:
Use separate reagents R1A, R1B, R2.

PRECAUTIONS

Reagent may contain some non-reactive and preservative components. It is suggested to handle carefully it, avoiding contact with skin and swallow.
Perform the test according to the general "Good Laboratory Practice" (GLP) guidelines.

SPECIMEN

Wine or any foodstuff once its utilization has been tested. Red wine samples can be analyzed without decolorizaton.

PROCEDURE 1

Wavelength: 340 nm
Lightpath: 1 cm
Temperature: 37°C

dispense:	reagent blank	standard	sample
reagent R1	2 ml	2 ml	2 ml
water	25 µl	-	-
standard	-	25 µl	-
sample	-	-	25 µl

Mix, incubate at 37°C for 3 minutes.
Read absorbances of standard (As_1) and sample (Ac_1) against reagent blank.

dispense:	reagent blank	standard	sample
reagent R2	500 µl	500 µl	500 µl

Mix, incubate at 37°C for 10 minutes.
Read absorbances of standard (As_2) and sample (Ac_2) against reagent blank.

PROCEDURE 2

Wavelength: 340 nm
Lightpath: 1 cm
Temperature: 37°C

dispense:	reagent blank	standard	sample
reagent R1A	1 ml	1 ml	1 ml
reagent R1B	1 ml	1 ml	1 ml
water	25 µl	-	-
standard	-	25 µl	-
sample	-	-	25 µl

Mix, incubate at 37°C for 3 minutes.
Read absorbances of standard (As_1) and sample (Ac_1) against reagent blank.

dispense:	reagent blank	standard	sample
reagent R2	500 µl	500 µl	500 µl

Mix, incubate at 37°C for 10 minutes.
Read absorbances of standard (As_2) and sample (Ac_2) against reagent blank.

RESULTS CALCULATION

$$\text{acetic acid g/l} = \frac{Ac_2 - (0.8 \times Ac_1)}{As_2 - (0.8 \times As_1)} \times \text{standard value}$$

TEST PERFORMANCE

Specificity
The method is specific for acetic acid.

Linearity
The method is linear up to 2 g/l.
If the limit value is exceeded, it is suggested to dilute the sample 1+4 with distilled water and to repeat the test, multiplying the result by 5.

Precision
White wine

intra-assay (n=10)	mean (g/l)	SD (g/l)	CV%
sample	0.224	0.003	1.11

inter-assay (n=20)	mean (g/l)	SD (g/l)	CV%
sample	0.223	0.007	3.07

Red wine

intra-assay (n=10)	mean (g/l)	SD (g/l)	CV%
sample	0.468	0.007	1.47

inter-assay (n=20)	mean (g/l)	SD (g/l)	CV%
sample	0.463	0.016	3.50

WASTE DISPOSAL

This product is made to be used in professional laboratories.
P501: Dispose of contents according to national/international regulations.

REFERENCES

H.U.Bergmeyer ed. 3, "Methods of enzymatic analysis" vol. VI pp. 639-645

MANUFACTURER

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SYMBOLS

LOT lot of manufacturing
REF code number
storage at temperature interval
expiration date (year/month)
warning, read enclosed documents
read the directions