ACETIC ACID
(LIQUID READY™)

PRODUCT INSTRUCTIONS

SKU: 700007708
K-ACETLQ

08/25

(50 Manual Assays per Kit) or
(500 Auto-Analyzer Assays per Kit)

Megazyme® by NEOGEN®

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FS02317_0825

INTRODUCTION:

Acetic Acid (acetate) occurs in a wide range of foods and beverages. In the wine industry, it is one of the most important quality parameters and is measured throughout the entire vinification process. 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 catalyzed 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. An alternative biochemistry is utilized in the Acetic Acid (Liquid Ready) test kit, based on the enzyme acetate kinase. This kit gives excellent linear calibration curves, and results in a stoichiometric change in absorbance due to the Acetic Acid present in the sample.

PRINCIPLE:

Acetate kinase (AK) in the presence of ATP converts Acetic Acid into acetyl-phosphate and adenosine-5'-diphosphate (ADP) (1). The ADP formed in (1) is reconverted into ATP and pyruvate, by phosphoenolpyruvate (PEP) in the presence of pyruvate kinase (PK) (2). In the presence of the enzyme D-lactate dehydrogenase (D-LDH), pyruvate is reduced to D-lactate by reduced nicotinamide-adenine dinucleotide (NADH) with the production of NAD^+ (3).

(1) $\text{Acetic Acid} + \text{ATP} \xrightarrow{\text{AK}} \text{Acetyl} - \text{phosphate} + \text{ADP}$

(2) $\text{ADP} + \text{PEP} \xrightarrow{\text{PK}} \text{ATP} + \text{Pyruvate}$

(3) $\text{Pyruvate} + \text{NADH} + \text{H}^+ \xrightarrow{\text{D-LDH}} \text{D} - \text{Lactic Acid} + \text{NAD}^+$

The amount of NAD^+ formed in this reaction pathway is stoichiometric with the amount of Acetic Acid. It is the NAD^+ which is measured by the decrease in absorbance at 340 nm.

SPECIFICITY, SENSITIVITY AND LINEARITY:

- The assay is specific for Acetic Acid.
- The kit provides two different methods based on the estimated Acetic Acid content in the sample: a High Range method for samples with higher concentrations and a Sensitive Range method for detecting lower concentrations.
- **High Range:** The limit of detection (LOD) is 0.011 g/L, and the limit of quantification (LOQ) is 0.033 g/L using a sample volume of 0.025 mL**.** The recommended measuring range is between 0.13 and 1.30 g/L using a sample volume of 0.025 mL. This corresponds to 3.25 µg – 32.5 µg of Acetic Acid per assay.
- **Sensitive Range:** The limit of detection (LOD) is 0.005 g/L, and the limit of quantification (LOQ) is 0.014 g/L using a sample volume of 0.1 mL. The recommended measuring range is between 0.033 and 0.33 g/L using a sample volume of 0.1 mL. This corresponds to 3.25 µg – 32.5 µg of Acetic Acid per assay.

INTERFERENCE:

Calcium chloride interferes at concentrations above 1 g/L. It is recommended that samples containing this interfering agent are diluted prior to testing.

SAFETY:

The general safety measures that apply to all chemical substances should be adhered to. After use, the reagents may be disposed of with standard laboratory waste, in accordance with local regulations and guidelines.

NOTE: For more information regarding the performance and safe usage of this product please refer to the associated validation report and SDS available from the Megazyme website.

KIT CONTENTS:

Kits are designed for use in both manual and automated workflows. The reagents are sufficient for performing 50 assays in manual format or 500 assays in auto-analyzer format. The kit contains:

Reagent 1 (2 x 50 mL):	ATP, PEP, NADH Ready to use. **Store at 4°C. See individual labels for expiry date.**
Reagent 2 (2 x 12.5 mL):	AK, PK, D-LDH Contains sodium azide (0.05% *w/v*) as a preservative. Ready to use. **Store at 4°C. See individual labels for expiry date.**
Standard (5 mL):	Acetic Acid standard (1.3 g/L) Contains sodium azide (0.02% *w/v*) as a preservative. Ready to use. **Store at 4°C. See individual labels for expiry date.**

NOTE: The Acetic Acid standard solution is only assayed where there is some doubt about the accuracy of the spectrophotometer being used or where it is suspected that inhibition is being caused by substances in the sample. The concentration of Acetic Acid is determined directly from the extinction coefficient of NAD^+.

PREPARATION OF REAGENT SOLUTIONS:

Bring all reagents to room temperature (20 - 25°C) before use.

MANUAL ASSAY PROCEDURE – HIGH RANGE:

Wavelength: 340 nm
Cuvette: 1 cm light path (glass or plastic)
Temperature: 20 - 25°C
Sample Volume: 0.025 mL
Final volume: 2.525 mL
Sample solution: 0.13 g/L to 1.3 g/L (i.e. 3.25 µg – 32.5 µg of Acetic Acid per cuvette)
Read against air (without a cuvette in the light path) or against water

Pipette into Cuvettes	Blank	Sample
Reagent 1	2.0 mL	2.0 mL
Sample	-	0.025 mL
Distilled Water	0.025 mL	-
Mix*, incubate for ~ 3 minutes at 20 - 25°C, then read the absorbances (A_1) Add Reagent 2 as described below:		
Reagent 2	0.5 mL	0.5 mL
Mix*, incubate for ~ 15 minutes at 20 -25°C, then read the absorbances (A_2). **		

* Either by aspiration with the pipette tip used to dispense the liquid or by gentle inversion after sealing the cuvette with a cuvette cap or Parafilm®.
** It may be necessary to check if the reaction has reached completion by continuing to read the absorbances at 1 minute intervals. If the reaction has not reached completion continue to measure absorbances until the values measured either remain the same, or increase constantly over 1 minute. If this "creep" rate is greater for the sample than for the blank, extrapolate the absorbances (sample and blank) back to the time of addition of Reagent 2.

NOTE: The reagent blank value must be determined once for each run and subtracted from each sample result.

MANUAL ASSAY PROCEDURE – SENSITIVE RANGE:

Wavelength: 340 nm
Cuvette: 1 cm light path (glass or plastic)
Temperature: 20 - 25°C
Sample Volume: 0.1 mL
Final volume: 2.6 mL
Sample solution: 0.033 g/L to 0.33 g/L (i.e. 3.25 µg – 32.5 µg of Acetic Acid per cuvette)
Read against air (without a cuvette in the light path) or against water

Pipette into Cuvettes	Blank	Sample
Reagent 1	2.0 mL	2.0 mL
Sample	-	0.1 mL
Distilled Water	0.1 mL	-
Mix*, incubate for ~ 3 minutes at 20 - 25°C, then read the absorbances (A_1) Add Reagent 2 as described below:		
Reagent 2	0.5 mL	0.5 mL
Mix*, incubate for ~ 15 minutes at 20 -25°C, then read the absorbances (A_2). **		

* Either by aspiration with the pipette tip used to dispense the liquid or by gentle inversion after sealing the cuvette with a cuvette cap or Parafilm®.
** It may be necessary to check if the reaction has reached completion by continuing to read the absorbances at 1 minute intervals. If the reaction has not reached completion continue to measure absorbances until the values measured either remain the same, or increase constantly over 1 minute. If this "creep" rate is greater for the sample than for the blank, extrapolate the absorbances (sample and blank) back to the time of addition of Reagent 2.

NOTE: The reagent blank value must be determined once for each run and subtracted from each sample result.

CALCULATION:

NOTE: These calculations can be simplified by using the *MegaCalc*™ tool, downloadable from the product page.

1. Calculation of the dilution factor (df)

Determine the dilution factor (df) based on the component ratios:

$$df = \frac{\text{Sample volume [mL]} + \text{R1 volume [mL]}}{\text{Total reaction volume [mL]}}$$

It follows for the Acetic Acid **High Range procedure**:

$$df = \frac{0.025 + 2.0}{2.525} = 0.802$$

It follows for the Acetic Acid **Sensitive Range procedure**:

$$df = \frac{0.1 + 2.0}{2.6} = 0.808$$

2. Calculation of the absorbance difference $\Delta A_{\text{Acetic Acid}}$

$$\Delta A_{\text{Acetic Acid}} = (A_1 \times df - A_2)_{\text{sample}} - (A_1 \times df - A_2)_{\text{blank}}$$

It follows for the Acetic Acid **High Range procedure**:

$$\Delta A_{\text{Acetic Acid}} = (A_1 \times 0.802 - A_2)_{\text{sample}} - (A_1 \times 0.802 - A_2)_{\text{blank}}$$

It follows for the Acetic Acid **Sensitive Range procedure**:

$$\Delta A_{\text{Acetic Acid}} = (A_1 \times 0.808 - A_2)_{\text{sample}} - (A_1 \times 0.808 - A_2)_{\text{blank}}$$

NOTE: Increasing or decreasing the sample volume with unchanged reagent volumes requires recalculation of the dilution factor; if volumes are changed, the system and performance may be affected.

3. Calculation of the Acetic Acid content in g/L

The concentration of Acetic Acid can be calculated as follows:

$$c = \frac{V \times MW}{\varepsilon \times d \times v} \times \Delta A_{\text{Acetic Acid}} \quad [g/L]$$

where:

V = final volume [mL]

MW = molecular weight of Acetic Acid [g/mol]

ε = extinction coefficient of NADH at 340 nm [l x mol^{-1} x cm^{-1}]

d = light path [cm]

v = sample volume [mL]

It follows for the Acetic Acid **High Range procedure**:

$$c = \frac{2.525 \times 60.05}{6300 \times 1.0 \times 0.025} \times \Delta A_{\text{Acetic Acid}} \quad [g/L]$$

$$= 0.9627 \times \Delta A_{\text{Acetic Acid}} \quad [g/L]$$

It follows for the Acetic Acid **Sensitive Range procedure**:

$$c = \frac{2.6 \times 60.05}{6300 \times 1.0 \times 0.1} \times \Delta A_{\text{Acetic Acid}} \quad [g/L]$$

$$= 0.2478 \times \Delta A_{\text{Acetic Acid}} \quad [g/L]$$

If the sample has been diluted during preparation, the result must be multiplied by the sample dilution factor, F.

4. Calculation of the Acetic Acid content in solid or semi-solid samples:

When analyzing solid and semi-solid samples which are weighed out for sample preparation, the content (g/100 g) is calculated from the amount weighed as follows:

$$\frac{C_{\text{Acetic Acid}}\ [g/L\ \text{sample solution}]}{\text{weight}_{\text{sample}}\ [g/L\ \text{sample solution}]} \times 100 \quad [g/100g]$$

AUTO-ANALYZER ASSAY PROCEDURE:

This kit has been designed for auto-analyzers and can be adapted to most instruments. A sample method is shown below (validated on the Awareness Technology, Inc. ChemWell®-T analyzer).

NOTE: For each batch of samples that is applied to the determination of Acetic Acid a calibration curve must be performed concurrently using the same batch of reagents.

Parameter	Details
Wavelength	340/405 nm (primary/secondary)
Temperature	20 - 37°C
Test	End-point test with following test sequence: – Add Reagent 1 **[0.2 mL]** – Add Sample or Calibrator **[0.01 mL]** – Pre-incubate 1-3 minutes [20 - 37°C] – Measure A_1 at 340/405 nm – Add Reagent 2 **[0.05 mL]** – Incubate 15 minutes at [20 - 37°C] – Measure A_2 at 340/405 nm – Calculate $A_2 - A_1$ against calibration curve
Calibration	Calibrate using 4 calibrators ranging from 0 – 0.325 g/L. The calibration curve is linear. An example of how to use the standard supplied with the kit to create a calibration curve is shown below: Calibrator 1 0 g/L (use distilled water) Calibrator 2 0.065 g/L (dilute Standard 20-fold) Calibrator 3 0.130 g/L (dilute Standard 10-fold) Calibrator 4 0.325 g/L (dilute Standard 4-fold) *Perform all dilutions with distilled water.*

SAMPLE PREPARATION:

1. Sample dilution

The kit provides two different methods based on the estimated Acetic Acid content in the sample: a High Range method for samples with higher concentrations (0.13 g/L to 1.3 g/L) and a Sensitive Range method for detecting lower concentrations (0.033 g/L to 0.33 g/L). The amount of Acetic Acid present in the sample should range from 0.033 g/L to 1.3 g/L.

Dilution Table (manual assay)

Estimated Concentration of Acetic Acid (g/L)	Dilution with Water	Dilution factor (F)
<0.33	No dilution required (Sensitive Range)	1
< 1.3	No dilution required (High Range)	1
1.3 -13	1 mL sample + 9 mL water (High Range)	10
13-130	1 mL sample + 99 mL water (High Range)	100

2. General sample preparation guide

- Clear, slightly colored, and approximately neutral, liquid samples at a concentration up to 1.3 g/L can be used directly in the assay.
- Turbid samples should be filtered or centrifuged.
- Acidic samples (pH < 3.0) must be neutralized to approximately pH 8.0.
- Samples containing carbon dioxide should be degassed by gentle agitation or stirring with a glass rod.
- Solid samples should be homogenized, extracted in water, and filtered or centrifuged if necessary.
- Strongly colored samples should be treated by the addition of 0.2 g of polyvinylpolypyrrolidone (PVPP) per 10 mL of sample in a tube. Shake the tube vigorously for 5 minutes and then filter through filter paper.
- Deproteinize samples and/or remove fat using the Carrez Clarification Kit (SKU; 700004270 K-CARREZ).

3. Suggested sample preparation examples

(a) Determination of Acetic Acid in wine. Pass through a 0.2 micron syringe filter to clarify. Alternatively, centrifuge an aliquot of wine for 5 minutes at 15,000 g.
Typically, a 5-fold dilution in distilled water is required for red wine (due to the coloring of the wine)
Typically, no dilution is required for white wine (Sensitive Range)

(b) Determination of Acetic Acid in vinegar: Pass through a 0.2 micron syringe filter to clarify. Alternatively, centrifuge an aliquot of vinegar for 5 minutes at 15,000 g. *Typically, a 500-fold dilution in distilled water is required (Sensitive Range).*

(c) Determination of Acetic Acid in fruit juices (e.g. apple juice): Pass through a 0.2 micron syringe filter to clarify. Alternatively, centrifuge an aliquot of juice for 5 minutes at 15,000 g. *Typically, no dilution is required (Sensitive Range).*

(d) Determination of Acetic Acid in cider: Remove carbonation by stirring a sample in a beaker for approximately 60 seconds using a glass rod. Pass through a 0.2 micron syringe filter and use the clear filtrate in the assay. *Typically, no dilution is required (Sensitive Range).*

(e) Determination of Acetic Acid in hard cheese (e.g. cheddar): Accurately weigh 2 g of ground cheese into a 100 mL volumetric flask and add 60 mL of distilled water. Incubate the flask at approx. 60°C for 20 minutes, with intermittent shaking. Cool the flask to 20-25°C and fill to the mark with distilled water. Store the flask at 4°C for 30 minutes and then filter using filter paper. Use the clear filtrate in the assay. *Typically, no dilution is required (Sensitive Range).*

(f) Determination of Acetic Acid in sour dressings and sauces (e.g. ketchup): Add 1 g of sample to a 100 mL volumetric flask and adjust the volume to 100 mL with distilled water. Store the solution at 4°C for 20 minutes to obtain separation of fat. Filter the solution using filter paper and use a clear filtrate for the assay. Typically, no further dilution is required (*Sensitive Range).*

(g) Determination of Acetic Acid in smoked salmon: Accurately weigh 10 g of smoked salmon, directly add to a blender followed by 100 mL of distilled water and blend for 30 seconds or until homogeneous. Filter the solution using filter paper and use a clear filtrate for the assay. *Typically, no dilution is required (High Range).*

IMPORTANT NOTE: The above are suggested sample preparation examples only. If you have questions about these or other matrices, please contact your local sales representative for support.

SERVICES AND TECHNICAL SUPPORT

Please reach out to your local sales representative should you require any assistance, particularly in relation to:

- Troubleshooting
- Data analysis
- Additional matrix testing
- Application support in relation to automated analyzers

Supporting documents can be found in the product page:

- Quick Reference Guide
- MegaCalc™
- Safety Data Sheets (SDS)
- Certificates Of Analysis (COA)
- Validation Report

Contact us for more information: neogen.com/contact

Without guarantee

The information contained in this assay protocol is, to the best of our knowledge, true and accurate, but since the conditions of use are beyond our control, no warranty is given or is implied in respect of any recommendation or suggestions which may be made or that any use will not infringe any patents.

User Responsibility:

- Users are responsible for familiarizing themselves with product instructions and information. Visit our website at neogen.com or contact your local Neogen® representative or authorized distributor for more information.
- When selecting a test method, it is important to recognize that external factors such as sampling methods, testing protocols, sample preparation, handling, laboratory technique and the sample itself may influence results.
- It is the user's responsibility in selecting any test method or product to evaluate a sufficient number of samples with the appropriate matrices and challenges to satisfy the user that the chosen test method meets the user's criteria.
- It is also the user's responsibility to determine that any test methods and results meet its customers' and suppliers' requirements.
- As with any test method, results obtained do not constitute a guarantee of the quality of the matrices or processes tested.

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