

ACETIC ACID *NOTE: New Standard Concentration*

Product #: **AA-F60 (30 Tests)**
AA-F150 (75 Tests)
AA-F500 (250 Tests)

Enzymatic, UV-Method

INTENDED USE

Acetic Acid FLEX-REAGENT™ is intended for measuring acetic acid (acetate) concentrations in wine.

KIT CONTENTS

	30T	75T	250T
Opti-Buffer Solution #1	20 mL	50 mL	170 mL
Co-Enzyme Powder #2	4 mL	10 mL	2x17 mL
MDH/CS Solution #3	1.3 mL	3.3 mL	2x5.5 mL
ACS Enzyme Powder #4a	1.3 mL	3.3 mL	2x5.5 mL
ACS Diluent #4b	1.4 mL	3.4 mL	12 mL
Acetic Acid 0.4G/L Std*	1 mL	1 mL	5 mL

* The 0.4G/L level standard is now supplied to improve calibration and monitoring at levels critical for winemaking. Previous kits contained 0.15G/L Standard; alternate standards and a 5-Level Set are available from Unitech Scientific. No change is required to Manual Calculations (extinction coefficient method) below.

SYSTEM REQUIREMENT

Spectrophotometer should be capable of reading 340 nm absorbance over a 0-2 A range with a 1 cm light path.

SAMPLES

If wine samples are visually clear, no sample pretreatment is needed. Filter or centrifuge turbid samples, e.g. juice, must or fermentation samples.

REAGENTS AND STORAGE

Kit contents are stable through the labeled expiration date when stored at 2-8 °C. The following are **ready to use**: Opti-Buffer, MDH/CS Solution, and Standard.

ASSAY PREPARATION

Dissolve Reagent Powders

1. Dissolve a bottle of **Co-Enzyme Powder (#2)** according to volume on label with deionized water.
2. Dissolve a bottle of **ACS Enzyme Powder (#4a)** with the labeled volume of **ACS Diluent (#4b)**.

Gently mix by inversion to dissolve. Write dissolution date on each label; these solutions are stable for **3 months** when stored at 2-8°C.

Prepare Working Reagent, manual method:
 (see **Appendix** for 'ChemWell-for-Wine™' Working Reagent)

Prepare Working Reagent just prior to testing, based on number of cuvettes (blank, standard and wine samples) in your assay, as below.

Each test requires:

Opti-Buffer Solution #1	0.67mL
Co-Enzyme Solution #2	0.133mL
Deionized Water	1.33mL
Working Rgt (Approx.)	2mL

Working Reagent is stable for 1-day at 5 °C. Allow W-Rgt to reach room temperature before use.

TESTING PROCEDURE

1. Pipet water into the Reagent Blank cuvette; pipet samples into respective cuvettes, pipet standard (or check wine/control) to monitor assay performance.
2. Pipet working reagent into cuvettes.

Pipette into Cuvettes	Reagent Blank Cuvette	Reaction Cuvettes
Sample, Controls		20µL
DI water	20µL	
Working Reagent	2 mL	2 mL
Mix and read absorbances (A ₀) at 340 nm		
MDH/CS Solution #3	40 uL (1 drop)	40 uL (1 drop)
Mix, wait 3 minutes, and read absorbances (A ₁)		
ACS Solution #4	40 uL (1 drop)	40 uL (1 drop)
Mix, wait 20-30 minutes, and read absorbances (A ₂).		

3. Zero spectrophotometer with Reagent Blank. Read Sample initial absorbance (A₀) values.
4. Add Solution #3, mix and wait 3 minutes and read Blank and Sample absorbance (A₁).
5. Add Solution #4, mix and wait 20 -30 minutes; read Blank and Sample absorbance (A₂).

The testing range is up to 0.75 G/L. If a calculated result is over-range, dilute sample and retest, multiply this test result by the dilution factor.

CALCULATIONS: The G/L result is calculated using a linearizing formula.

1. Flex Calculator™ Method:

Our online "Flex Calculator™ Acetic Acid" spreadsheet at <http://unitechscientific.com/calculators.htm> is available for download. ΔA_{AcAc} and G/L values will be calculated automatically.

2. Manual Calculation Method - Calculate ΔA_{AcAc} and G/L:

a) $\Delta A_{AcAc} =$

$$\left[\frac{(A_2 - A_0)_{\text{sample}} - (A_1 - A_0)_{\text{sample}}^2}{(A_2 - A_0)_{\text{sample}}} \right] - \left[\frac{(A_2 - A_0)_{\text{blank}} - (A_1 - A_0)_{\text{blank}}^2}{(A_2 - A_0)_{\text{blank}}} \right]$$

b) **Acetic Acid G/L = 1.001 ΔA_{AcAc}** (Refer to APPENDIX)

SPECIFICITY

This method is specific for acetate in wine samples; ethyl acetate is largely undetected by enzymatic analysis. For samples with unusually high ethyl acetate content, a correction method is described in our "Flex Calculator™ Acetic Acid" spreadsheet.

QUALITY CONTROL

Assay the acetic acid standard in each test to monitor reaction completion and assay performance. The recovered value should be within 15% of the labeled value. Factors that may affect the performance of this test include instrument function, temperature, glassware cleanliness, and pipetting accuracy (use calibrated micro-pipet). A 5-Level AA Standards Kit is available from Unitech Scientific.

APPENDIX

NOTES FOR MANUAL CALCULATIONS **Extinction Coefficient:** (G/L results are based on factor and the ΔA_{AcAc} values above. Compare standard result with known value to verify recovery.) Factor is derived as follows:

$$G/L = \frac{MW \times T.V. \times d.f. \times \Delta A_{Acetic\ Acid}}{(\epsilon)(P)(1000mg/g)(SV)} = 1.001\Delta A_{AcAc}$$

MW = 60.05 G/mole

TV = total reaction vol. (2+ 0.02+0.04+0.04) = 2.10 mL

SV = sample volume (0.020 mL)

ϵ (absorptivity of NADP) = 6.3 @334-340nm [or 3.4@365nm]

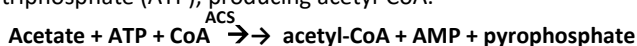
P = 1 cm light path

d.f. = dilution factor

Adjust calculations if alternate Working Reagent & Sample Volumes are used.

METHODOLOGY & CHEMICAL PRINCIPLES

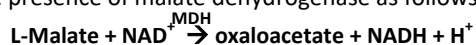
The enzyme acetyl-CoA synthetase (ACS) catalyzes the reaction of AA (acetate) with coenzyme A (CoA) in the presence of adenosine-5'-triphosphate (ATP), producing acetyl-CoA.¹



Citrate synthase (CS) catalyzes the reaction of acetyl-CoA and oxaloacetate to form citrate.



Oxaloacetate, consumed in this reaction, is formed from L-Malic acid in the presence of malate dehydrogenase as follows:



The increase in NADH concentration is measured at 340nm and is the basis for calculation of AA concentration in the sample.

SIGNIFICANCE OF MEASUREMENTS

Acetic acid is the primary acid formed during wine spoilage; legal limits vary by wine region. Volatile acidity determined by distillation consists primarily of AA, propionic and lactic acids; specific (enzymatic) acetic acid values are 90-98% of volatile acidity values in modern winemaking practice.

Ethyl acetate is typically determined using GC-FID, and is largely undetected by enzymatic and distillation analysis.

following:

1. Working Reagent.

	20T	55T
Opti-Buffer Solution #1	2.0 mL	4.0 mL
MDH/CS Solution #3	0.13 mL	0.26 mL
Deionized Water	<u>4.0 mL</u>	<u>8.0 mL</u>
WRgt (Approx. Total)	6.1 mL	12.2 mL

(# of Tests accounts for Reagent Bottle dead volume)

This Working Reagent is stable for 5-days at 2-8 °C.

2. Co-Enzyme **ChemWell for Wine, CW-T for Wine**
Dissolve Co-enzyme powder as described in **ASSAY PREPARATION (*)**. Place this Co-enzyme Solution in your Reagent Rack.

3. ACS Enzyme

Dissolve the ACS enzyme powder as described in **ASSAY PREPARATION** using ACS Diluent (*).

(*) Write dissolution date on each label; Co-A and ACS Solutions are stable for **3 months** when stored at 2-8°C.

ChemWell for Wine (uses diluted ACS)

Prepare a 6-fold dilution of ACS Enzyme Solution #4, per table below, and place Diluted ACS in Reagent Rack:

	20T	55T
Deionized Water	1.0mL	1.5mL
ACS Enzyme Solution #4	0.2mL	0.3mL
Diluted ACS (Approx. Total)	1.2mL	1.8mL

Diluted ACS Enzyme is stable for **1 day at 5°C, prepare fresh daily.**

ChemWell-T for Wine (no ACS dilution)

Place an aliquot of the dissolved ACS enzyme (ACS Powder + ACS Diluent) – no Water Dilution - directly into the Reagent Rack.

(If this is a change in procedure in your lab, to assure you have the latest protocol, contact Unitech Technical Service.)

AUTOMATION CALCULATIONS (Standard Method)

'ChemWell for Wine' calculates results automatically from either one standard or a multi-point standard curve; dilutes and retests values above linear range. Multi-Point Standard curve - G/L acetic acid values are read from a curve as follows: $\Delta A = A_2 - A_1$ are calculated for Blank, Standards, and Samples. ΔA are corrected to give "Net ABS" Values (ΔA Blank is subtracted from - ΔA for Standards and Samples.) Net Values are plotted vs. concentration for each standard and sample G/L are read from the curve.

TRADEMARKS:

"ChemWell for Wine", "Flex Calculator", "FLEX Reagent" are Trademarks of Unitech Scientific LLC

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AUTOMATED TESTING ChemWell for Wine, CW-T for Wine
ChemWell acetic acid analysis range is up to 1.5 G/L, and uses the